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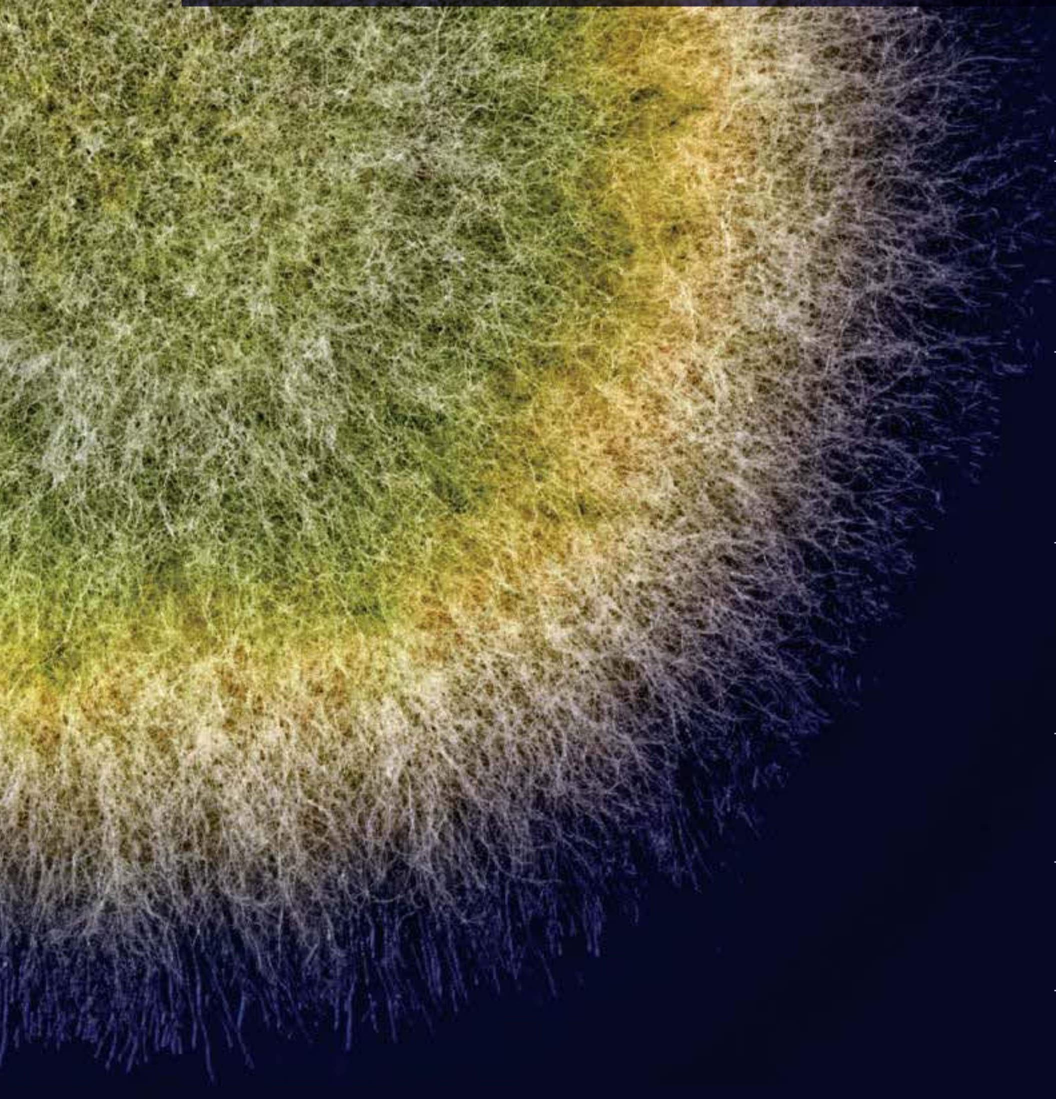

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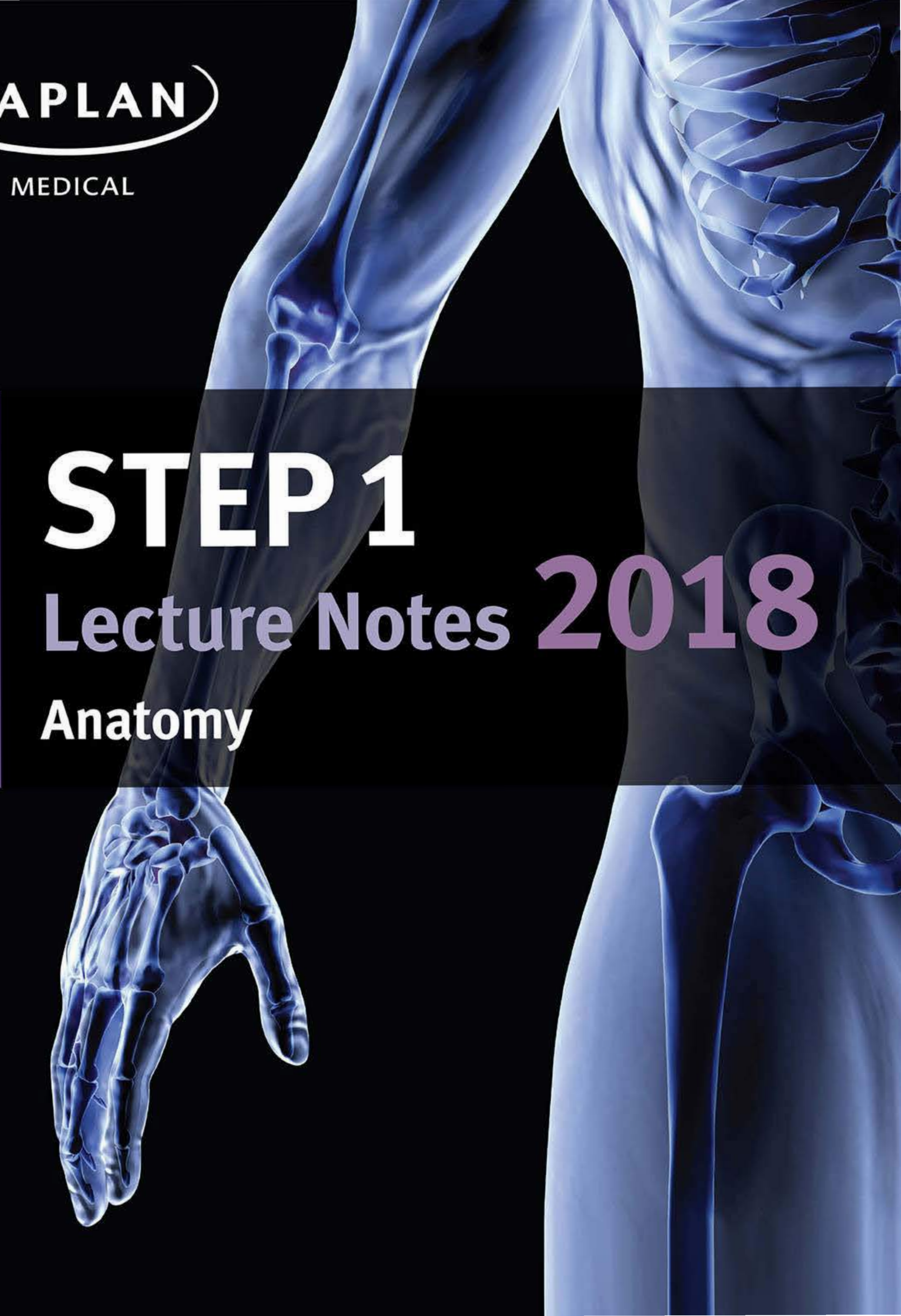

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PART I

Early Embryology and Histology: Epithelia

Gonad Development 1

Learning Objectives

- ❑ Explain information related to indifferent gonad
- ❑ Interpret scenarios on testis and ovary
- ❑ Answer questions about meiosis
- ❑ Interpret scenarios on spermatogenesis
- ❑ Solve problems concerning oogenesis

INDIFFERENT GONAD

Although sex is determined at fertilization, the gonads initially go through an **indifferent stage** weeks 4–7 when there are no specific ovarian or testicular characteristics. The indifferent gonads develop in a longitudinal elevation or ridge of **intermediate mesoderm** called the **urogenital ridge**. The components of the indifferent gonads are as follows:

- **Primordial germ cells** provide a critical inductive influence on gonad development, migrating in at week 4. They arise from the lining cells in the wall of the yolk sac.
- **Primary sex cords** are finger-like extensions of the surface epithelium which grow into the gonad that are populated by the migrating primordial germ cells.
- **Mesonephric (Wolffian)** and the **paramesonephric (Mullerian)** ducts of the indifferent gonad contribute to the male and female genital tracts, respectively.

TESTIS AND OVARY

The indifferent gonad develops into either the testis or ovary.

Development of the **testis and male reproductive system** is directed by the following:

- **Sry gene** on the short arm of the **Y chromosome**, which encodes for **testis-determining factor** (TDF)
- **Testosterone,** which is secreted by the **Leydig cells**
- **Müllerian-inhibiting factor (MIF),** which is secreted by the **Sertoli cells**
- **Dihydrotestosterone (DHT):** external genitalia

Development of the **ovary and female reproductive system** requires estrogen. Ovarian development occurs in the absence of the Sry gene and in the presence of the WNT4 gene.

MIF: Müllerian-inhibiting factor
TDF: testis-determining factor

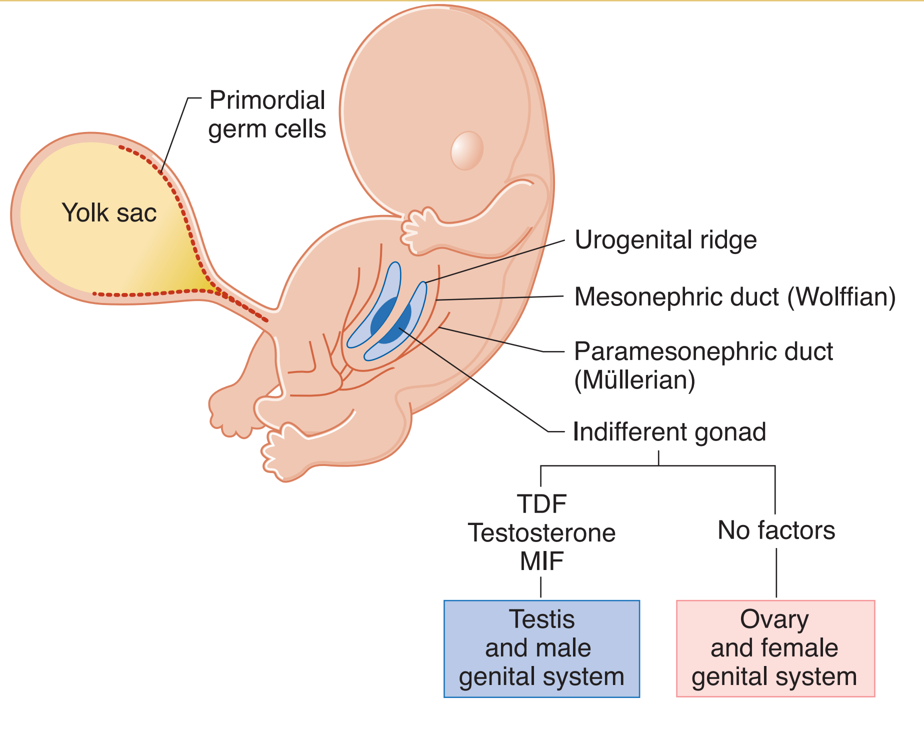

Figure I-1-1. Development of Testis and Ovary

MEIOSIS

Meiosis occurs within the testis and ovary. This is a specialized process of cell division that produces the male gamete **(spermatogenesis)** and female gamete **(oogenesis)**. There are notable differences between spermatogenesis and oogenesis.

Meiosis consists of 2 cell divisions. In **meiosis I**, the following events occur:

- **Synapsis:** pairing of 46 homologous chromosomes
- **Crossing over:** exchange of segments of DNA
- **Disjunction:** separation of 46 homologous chromosome pairs (no centromere-splitting) into 2 daughter cells, each containing 23 chromosome pairs

In **meiosis II,** synapsis does not occur, nor does crossing over. Disjunction does occur with centromere-splitting.

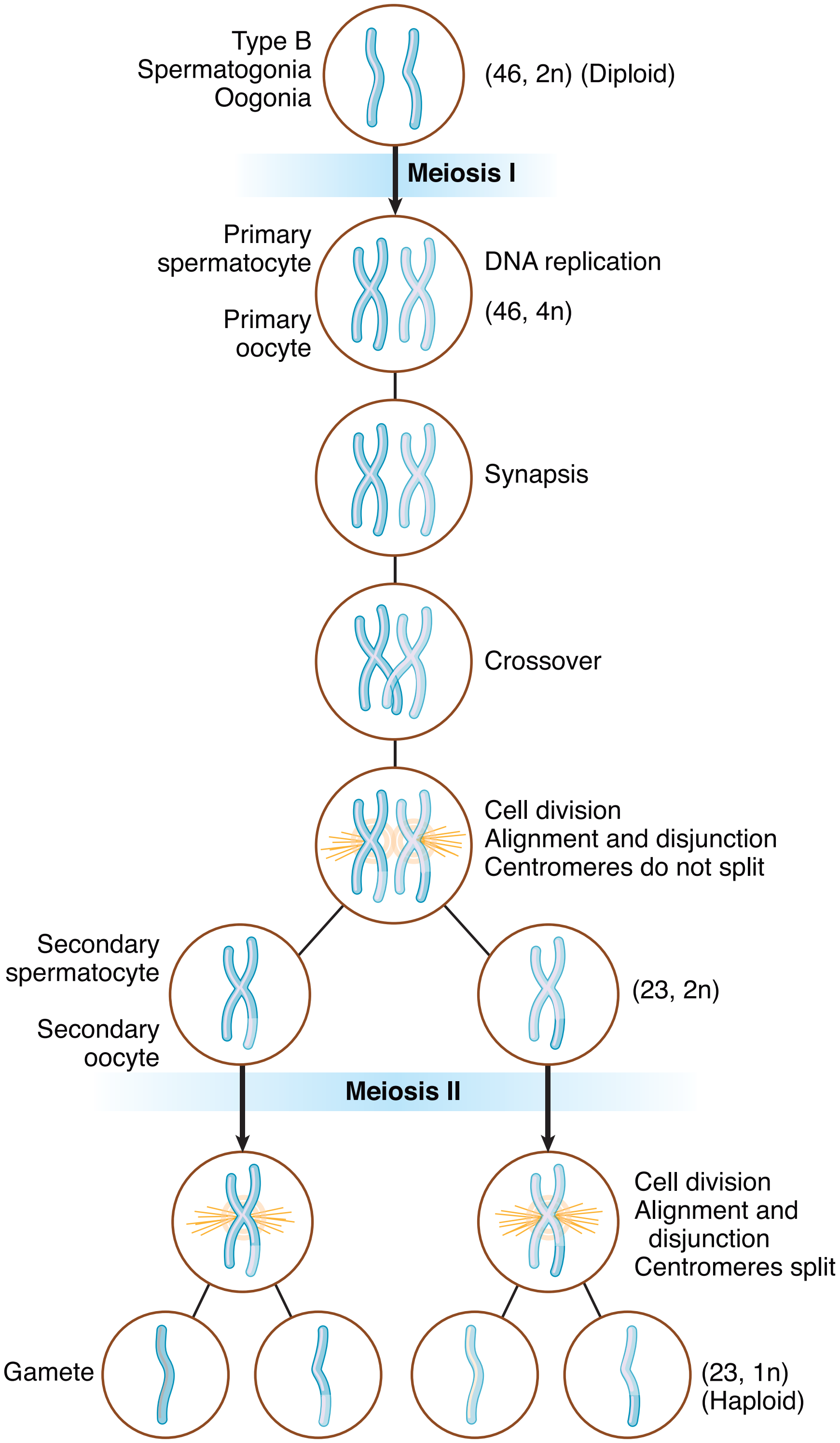

Figure I-1-2. Meiosis

SPERMATOGENESIS

At week 4, primordial germ cells arrive in the indifferent gonad and remain **dormant until puberty**.

- When a boy reaches puberty, primordial germ cells differentiate into **type A spermatogonia,** which serve as stem cells throughout adult life.
- Some type A spermatogonia differentiate into **type B spermatogonia.**
- Type B spermatogonia enter meiosis I to form **primary spermatocytes.**
- Primary spermatocytes form 2 **secondary spermatocytes**.
- Secondary spermatocytes enter meiosis II to form 2 **spermatids.**
- Spermatids undergo **spermiogenesis,** which is a series of morphological changes resulting in the mature **spermatozoa**.

OOGENESIS

At week 4, primordial germ cells arrive in the indifferent gonad and differentiate into oogonia. Oogonia enter meiosis I to **form primary oocytes**. All primary oocytes are formed by **month 5 of fetal life**; they are **arrested the first time in prophase (diplotene) of meiosis** I and remain arrested until puberty.

- Primary oocytes arrested in meiosis I are present at birth.
- When a girl reaches puberty, during each monthly cycle a primary oocyte becomes unarrested and completes meiosis I to form a secondary oocyte and polar body.
- The secondary oocyte becomes **arrested the second time in metaphase of meiosis II** and is ovulated.
- At fertilization within the uterine tube, the secondary oocyte completes meiosis II to form a **mature oocyte** and **polar body.**

First 8 Weeks of Development

2

Learning Objectives

- ❑ Solve problems concerning beginning of development
- ❑ Demonstrate understanding of the formation of the bilaminar embryo
- ❑ Solve problems concerning embryonic period

WEEK 1: BEGINNING OF DEVELOPMENT

Fertilization occurs in the **ampulla of the uterine tube** when the male and female pronuclei fuse to form a **zygote**. At fertilization, the secondary oocyte rapidly completes meiosis II.

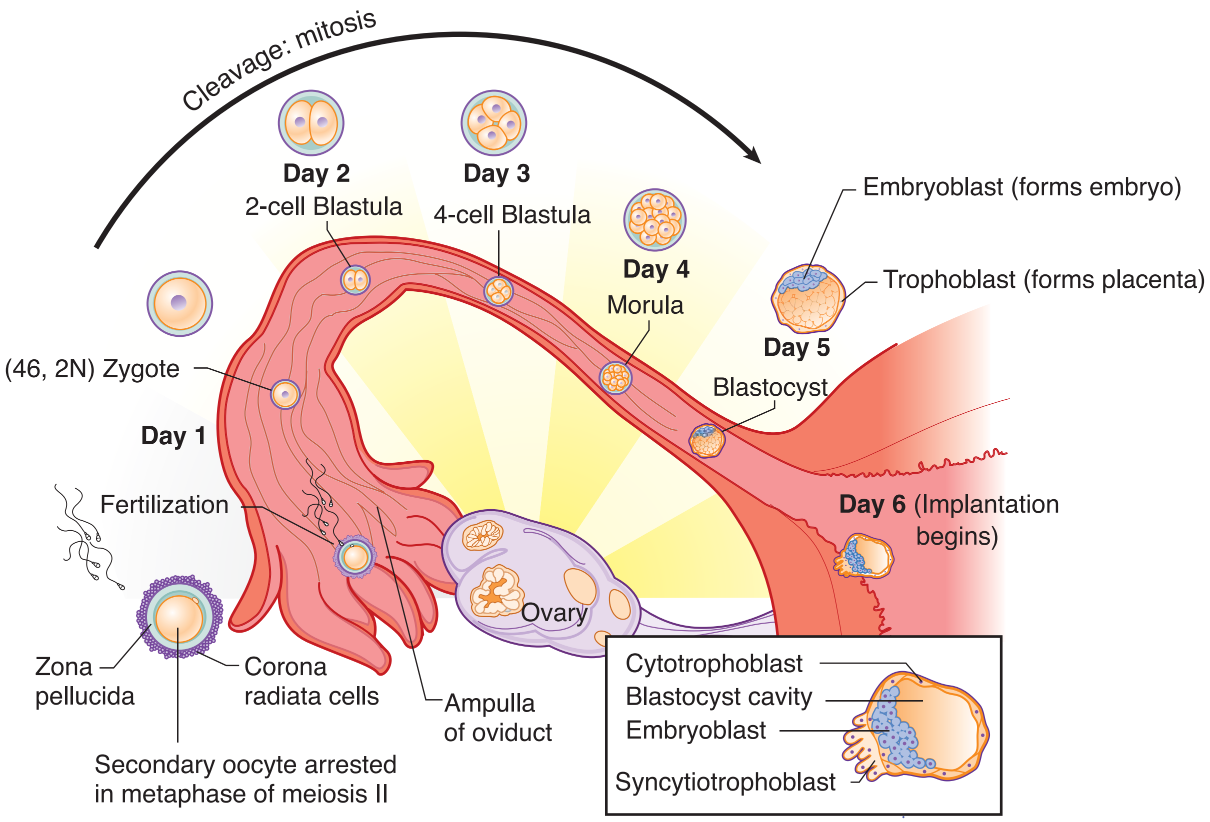

Figure I-2-1. Week 1

Prior to fertilization, spermatozoa undergo 2 changes in the female genital tract:

- **Capacitation** consists of the removal of several proteins from the plasma membrane of the acrosome of the spermatozoa. It occurs over about 7 hours in the female reproductive tract.
- Hydrolytic enzymes are released from the acrosome used by the sperm to penetrate the zona pellucida. This results in a **cortical reaction** that prevents other spermatozoa penetrating the zona pellucida thus preventing polyspermy.

During the first 4–5 days of week 1, the zygote undergoes rapid mitotic division (**cleavage**) in the oviduct to form a **blastula**, consisting of increasingly smaller **blastomeres**. This becomes the **morula** (32-cell stage).

A **blastocyst** forms as fluid develops in the morula. The blastocyst consists of an inner cell mass known as the **embryoblas**t, and the outer cell mass known as the **trophoblast**, which becomes the placenta.

At the end of week 1, the trophoblast differentiates into the **cytotrophoblast** and **syncytiotrophoblast** and then implantation begins.

Clinical Correlate

Ectopic Pregnancy

Tubal (most common form) usually occurs when the blastocyst **implants within the ampulla** of the uterine tube because of delayed transport. Risk factors include endometriosis, pelvic inflammatory disease, tubular pelvic surgery, and exposure to diethylstilbestrol (DES.) Clinical signs include abnormal or brisk uterine bleeding, sudden onset of abdominal pain that may be confused with appendicitis, missed menstrual period (e.g., LMP 60 days ago), positive human chorionic gonadotropin test, culdocentesis showing intraperitoneal blood, and positive sonogram.

Abdominal form usually occurs in the **rectouterine pouch** (pouch of Douglas).

Implantation

For implantation to occur, the **zona pellucida** must degenerate. The blastocyst usually implants within the **posterior wall of the uterus.** The embryonic pole of blastocyst implants first. The blastocyst implants within the **functional layer** of the endometrium during the **progestational phase** of the menstrual cycle.

WEEK 2: FORMATION OF THE BILAMINAR EMBRYO

In week 2, the embryoblast differentiates into the **epiblast** and **hypoblast**, forming a **bilaminar embryonic disk**. The epiblast forms the **amniotic cavity** and hypoblast cells migrate to form the **primary yolk sac**. The **prechordal plate**, formed from fusion of epiblast and hypoblast cells, is the site of the future **mouth**.

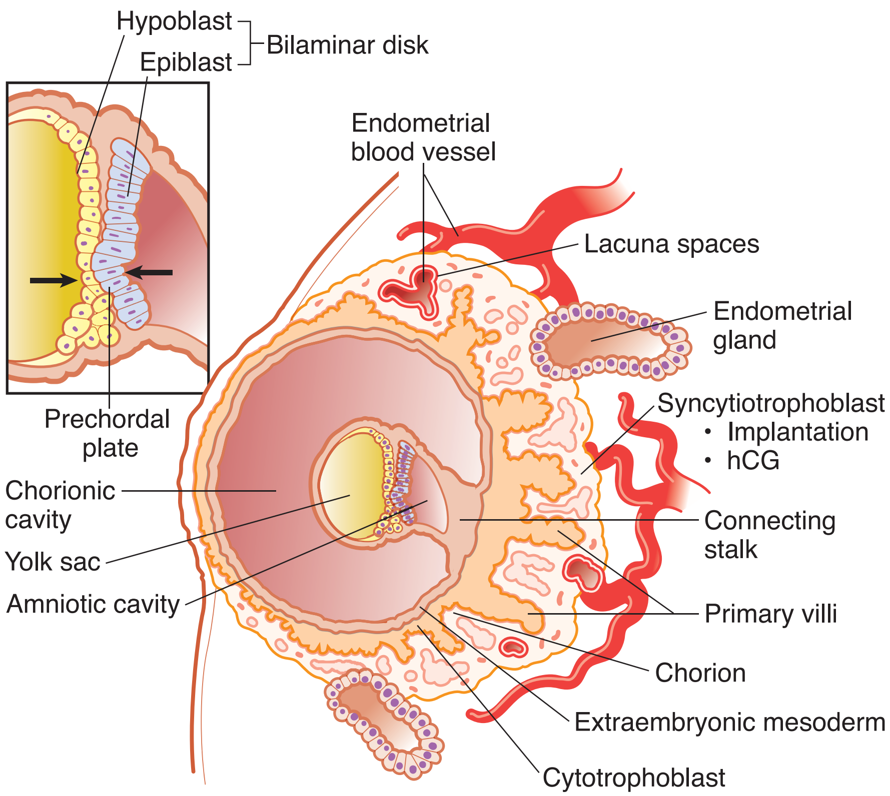

Figure I-2-2. Week 2

Extraembryonic mesoderm is derived from the epiblast. **Extraembryonic somatic mesoderm** lines the cytotrophoblast, forms the connecting stalk, and covers the amnion. **Extraembryonic visceral mesoderm** covers the yolk sac.

The connecting stalk suspends the conceptus within the chorionic cavity. The wall of the chorionic cavity is called the **chorion**, consisting of extraembryonic somatic mesoderm, the cytotrophoblast, and the syncytiotrophoblast.

The **syncytiotrophoblast** continues its growth into the endometrium to make contact with endometrial blood vessels and glands. **No mitosis occurs in the syncytiotrophoblast.** The cytotrophoblast is mitotically active.

Hematopoiesis occurs initially in the mesoderm surrounding the yolk sac (up to 6 weeks) and later in the fetal liver, spleen, thymus (6 weeks to third trimester), and bone marrow.

Clinical Correlate

Human chorionic gonadotropin (hCG), a glycoprotein produced by the syncytiotrophoblast, stimulates progesterone production by the corpus luteum. hCG can be assayed in maternal blood or urine and is the basis for early pregnancy testing. hCG is detectable throughout pregnancy.

- Low hCG level may predict a spontaneous abortion or ectopic pregnancy.
- High hCG level may predict a multiple pregnancy, hydatidiform mole, or gestational trophoblastic disease.

WEEKS 3–8: EMBRYONIC PERIOD

All major organ systems begin to develop during the **weeks 3–8**. By the end of this period, the embryo begins to look human, and the nervous and cardiovascular systems start to develop. Week 3 corresponds to the first missed menstrual period.

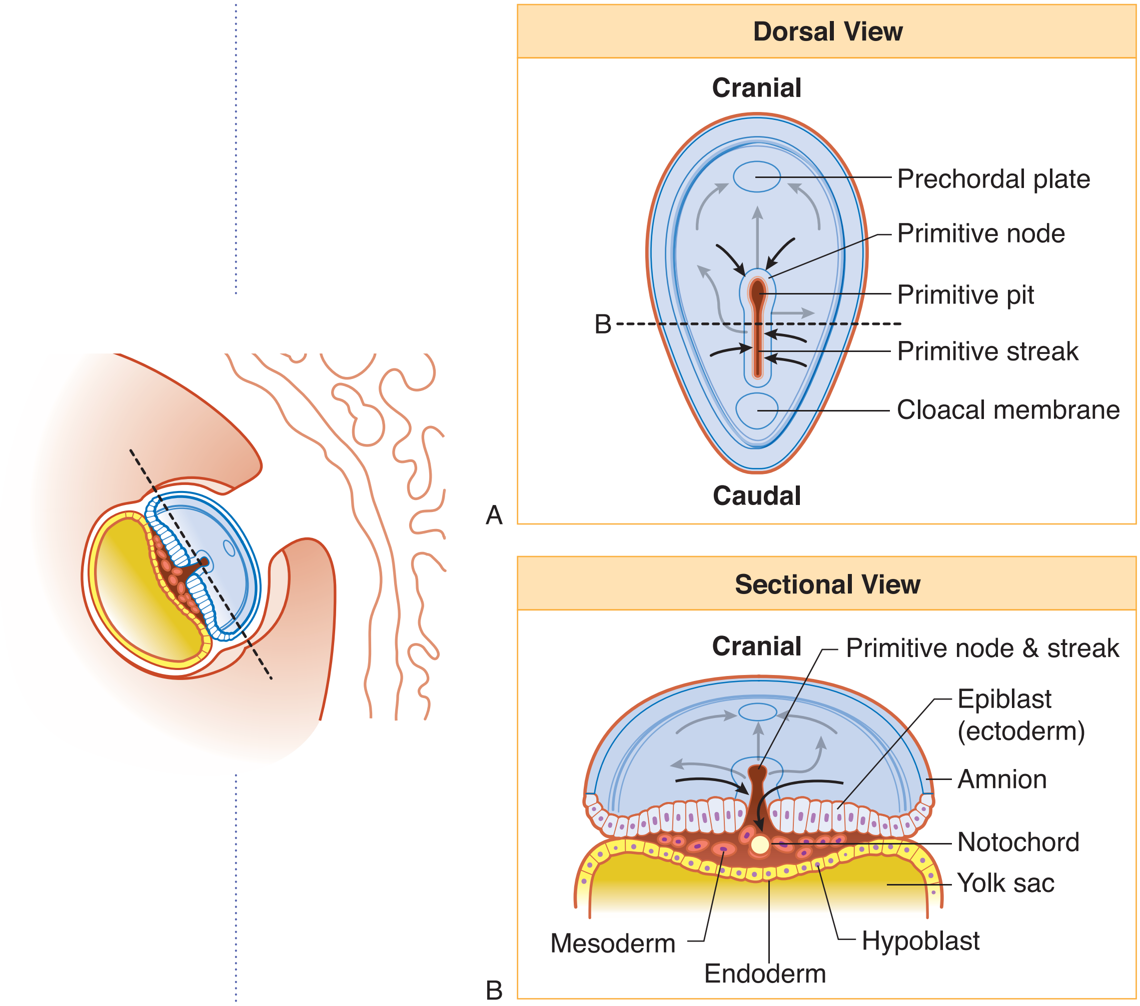

Figure I-2-3. Week 3

During this time **gastrulation** also takes place; this is the process by which the 3 primary germ layers are produced: **ectoderm, mesoderm**, and **endoderm**. It begins with the formation of the **primitive streak** within the epiblast.

- Ectoderm forms **neuroectoderm** and **neural crest cells.**
- Mesoderm forms **paraxial mesoderm** (35 pairs of somites), **intermediate mesoderm**, and **lateral mesoderm.**

Clinical Correlate

Sacrococcygeal teratoma: a tumor that arises from remnants of the primitive streak; often contains various types of tissue (bone, nerve, hair, etc)

Chordoma: a tumor that arises from remnants of the notochord, found either intracranially or in the sacral region

Hydatidiform mole: results from the partial or complete replacement of the trophoblast by dilated villi

- In a **complete mole, there is no embryo;** a haploid sperm fertilizes a blighted ovum and reduplicates so that the karyotype is 46,XX, with all chromosomes of paternal origin. In a **partial mole,** there is a haploid set of maternal chromosomes and usually 2 sets of paternal chromosomes so that the typical karyotype is 69,XXY.
- **Molar pregnancies have high levels of hCG, and 20% develop into a malignant trophoblastic disease, including choriocarcinoma.**

Table I-2-1. Germ Layer Derivatives

Ectoderm	Mesoderm	Endoderm
Surface ectoderm Epidermis Hair Nails Inner ear, external ear Enamel of teeth Lens of eye Anterior pituitary (Rathke's pouch) Parotid gland Anal canal below pectinate line **Neuroectoderm** Neural tube Central nervous system Retina and optic nerve Pineal gland Neurohypophysis Astrocytes Oligodendrocytes **Neural crest ectoderm** Adrenal medulla Ganglia Sensory—Pseudounipolar Neurons Autonomic—Postganglionic Neurons Pigment cells Schwann cells Meninges Pia and arachnoid mater Pharyngeal arch cartilage Odontoblasts Parafollicular (C) cells Aorticopulmonary septum Endocardial cushions	**Muscle** Smooth Cardiac Skeletal Connective tissue All serous membranes Bone and cartilage Blood, lymph, cardiovascular organs Adrenal cortex Gonads and internal reproductive organs Spleen Kidney and ureter Dura mater **Notochord** Nucleus pulposus	**Forms epithelial lining of:** GI track: foregut, midgut, and hindgut Lower respiratory system: larynx, trachea, bronchi, and lung Genitourinary system: urinary bladder, urethra, and lower vagina Pharyngeal pouches: • Auditory tube and middle ear • Palatine tonsils • Parathyroid glands • Thymus **Forms parenchyma of:** • Liver • Pancreas • Submandibular and sublingual glands • Follicles of thyroid gland
		Extra embryonic structures Yolk sac derivatives: Primordial germ cells Early blood cells and blood vessels

Histology: Epithelia

3

Learning Objectives

- ❑ Demonstrate understanding of epithelial cells
- ❑ Use knowledge of epithelium
- ❑ Interpret scenarios on cytoskeletal elements
- ❑ Explain information related to cell adhesion molecules
- ❑ Answer questions about cell surface specializations

Histology is the study of normal tissues. Groups of cells make up tissues, tissues form organs, organs form organ systems, and systems make up the organism. Each organ consists of 4 types of tissue: epithelial, connective, nervous, and muscular.

Only certain aspects of epithelia will be reviewed here; other aspects of histology are reviewed elsewhere in this book.

EPITHELIAL CELLS

Epithelial cells are often polarized: the structure, composition, and function of the apical surface membrane differ from those of the basolateral surfaces. The polarity is established by the presence of tight junctions that separate these 2 regions. Internal organelles are situated symmetrically in the cell. Membrane polarity and tight junctions are essential for the transport functions of epithelia.

Many simple epithelia transport substances from one side to the other (kidney epithelia transport salts and sugars; intestinal epithelia transport nutrients, antibodies, etc.). There are 2 basic mechanisms used for these transports:

- Transcellular pathway through which larger molecules and a combination of diffusion and pumping in the case of ions that pass through the cell
- Paracellular pathway that permits movement between cells

Tight junctions regulate the paracellular pathway, because they prevent backflow of transported material and keep basolateral and apical membrane components separate.

Epithelial polarity is essential to the proper functioning of epithelial cells; when polarity is disrupted, disease can develop. For example, epithelia lining the trachea, bronchi, intestine, and pancreatic ducts transport chloride from basolateral surface to lumen via pumps in the basolateral surface and channels in

the apical surface. The transport provides a driving force for Na by producing electrical polarization of the epithelium. Thus NaCl moves across, and water follows. In cystic fibrosis the apical Cl channels do not open. Failure of water transport results in thickening of the mucous layer covering the epithelia.

Transformed cells may lose their polarized organization, and this change can be easily detected by using antibodies against proteins specific for either the apical or basolateral surfaces. Loss of polarity in the distribution of membrane proteins may eventually become useful as an early index of neoplasticity.

Epithelia are always lined on the basal side by connective tissue containing blood vessels. Since epithelia are avascular, interstitial tissue fluids provide epithelia with oxygen and nutrients.

Epithelia modify the 2 compartments that they separate. The epithelial cells may either secrete into or absorb from each compartment, and may selectively transport solutes from one side of the barrier to the other.

Epithelia renew themselves continuously, some very rapidly (skin and intestinal linings), some at a slower rate. This means that the tissue contains stem cells that continuously proliferate. The daughter cells resulting from each cell division either remain in the pool of dividing cells or differentiate.

The epithelial subtypes are as follows:

- Simple cuboidal epithelium (e.g., renal tubules, salivary gland acini)
- Simple columnar epithelium (e.g., small intestine)
- Simple squamous epithelium (e.g., endothelium, mesothelium, epithelium lining the inside of the renal glomerular capsule)
- Stratified squamous epithelium: nonkeratinized (e.g., esophagus) and keratinizing (e.g., skin)
- Pseudostratified columnar epithelium (e.g., trachea, epididymis)
- Transitional epithelium (urothelium) (e.g., ureter and bladder)
- Stratified cuboidal epithelium (e.g., salivary gland ducts)

EPITHELIUM

Hematoxylin-and-Eosin Staining

The most common way to stain tissues for viewing in the light microscope is to utilize hematoxylin-and-eosin (H&E) staining.

Hematoxylin is a blue dye which stains basophilic substrates that are the acidic cellular components such as DNA and RNA. Hematoxylin stains nuclei blue, and may tint the cytoplasm of cells with extensive mRNA in their cytoplasm.

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Figure I-3-1. Kidney tubule simple cuboidal epithelium (arrow) stained with H&E, L-lumen

Eosin is a pink-to-orange dye which stains acidophilic substrates such as basic components of most proteins. Eosin stains the cytoplasm of most cells and many extracellular proteins, such as collagen, pink.

Epithelial Subtypes

Simple columnar epithelium is found in the small and large intestine.

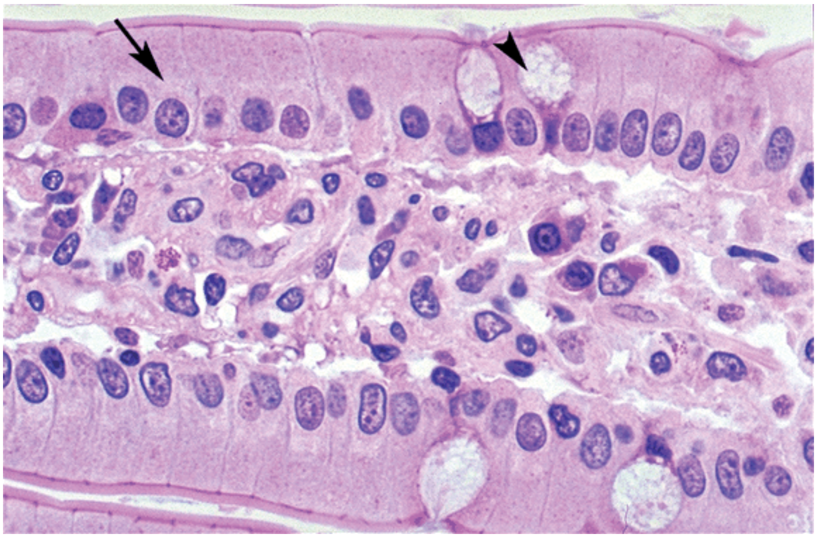

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Figure I-3-2. Small Intestine Simple Columnar Epithelium Enterocytes (arrow), Goblet cells (arrowhead)

Simple squamous epithelium forms an endothelium that lines blood vessels, a mesothelium that forms part of a serous membrane or forms the epithelium lining of the inside of the renal glomerular capsule.

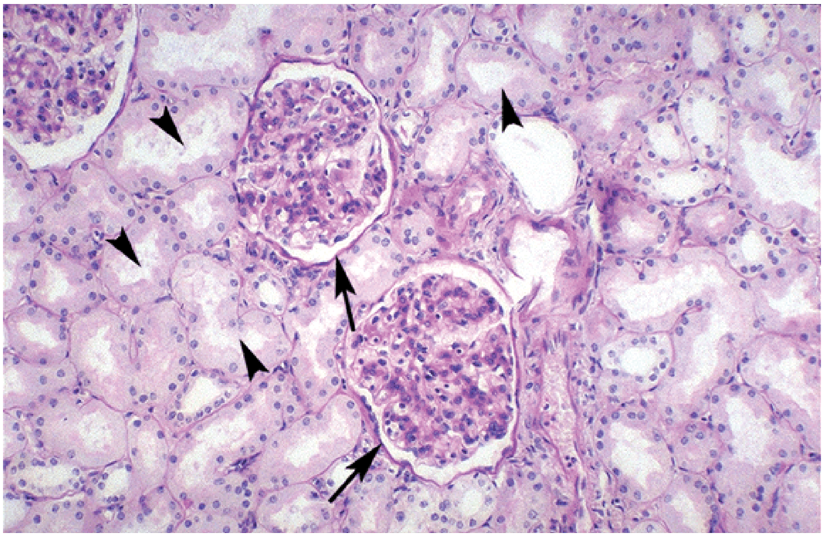

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Figure I-3-3. Kidney simple squamous epithelium (arrows), simple cuboidal epithelium (arrowheads)

Pseudostratified columnar epithelium is found in the nasal cavity, trachea, bronchi, and epididymis.

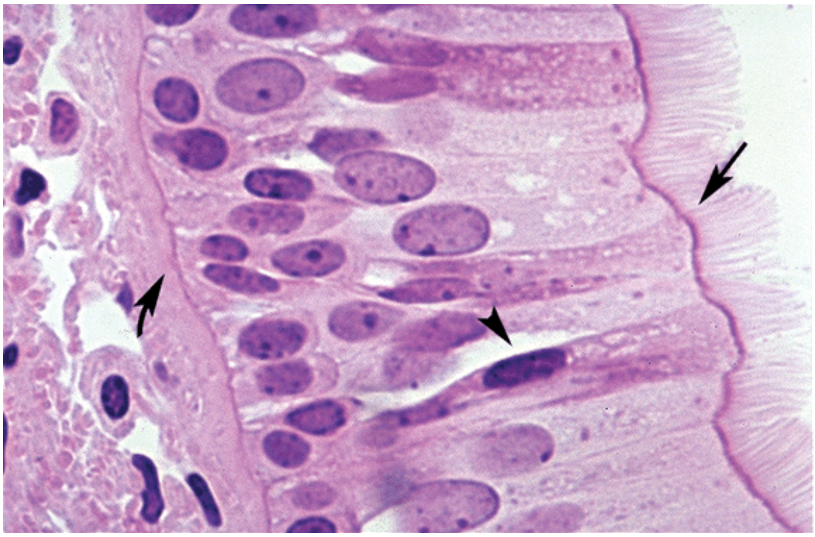

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Figure I-3-4. Trachea pseudostratified columnar epithelium with true cilia (arrow) and goblet cells (arrowhead), basement membrane (curved arrow)

Transitional epithelium is found in the ureter and bladder.

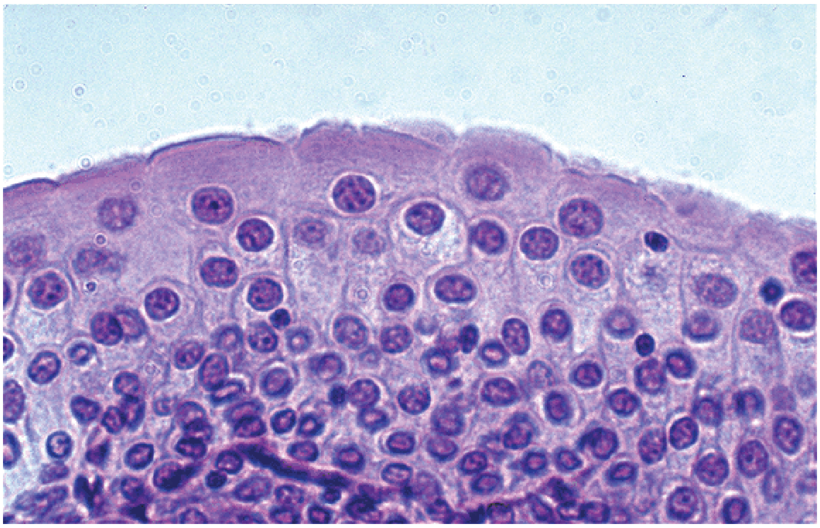

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Figure I-3-5. Bladder Transitional Epithelium

Stratified squamous epithelium is found in the oral cavity, pharynx, and esophagus (non-keratinized) and in the skin (keratinizing).

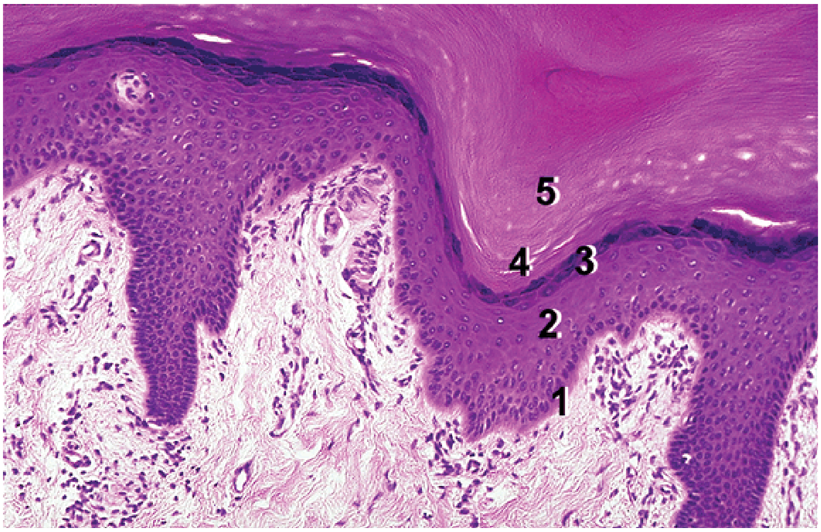

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Figure I-3-6. Stratified Squamous Epithelium (Thick Skin)
(1) stratum basale (2) stratum spinosum (3) stratum granulosum
(4) stratum lucidum (5) stratum corneum

Simple cuboidal epithelium is the epithelium of the renal tubules and the secretory cells of salivary gland acini.

Stratified cuboidal epithelium is found in the ducts of salivary glands.

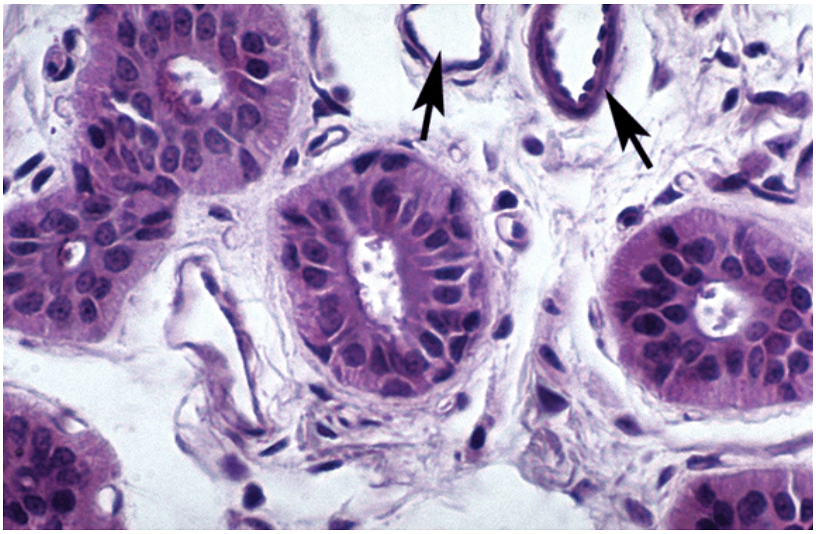

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Figure I-3-7. Ducts of salivary gland with stratified cuboidal epithelium small blood vessels with endothelium and smooth muscle (arrows)

Glands

Unicellular glands are goblet cells found in the respiratory and GI epithelium. **Multicellular glands** may be exocrine (salivary gland) or endocrine (thyroid gland), but all have tubules or acini formed mainly by a simple cuboidal epithelium. Only exocrine glands have ducts, which serve as conduits for glandular secretions to a body surface or to a lumen.

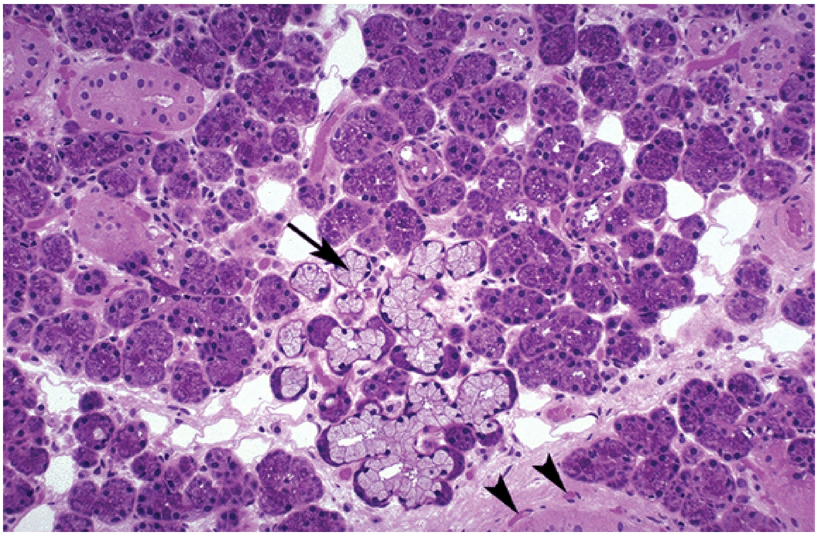

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Figure I-3-8. Submandibular Gland

Mixed salivary gland with mucous acini (arrow) and darker staining serous acini; small blood vessels (arrowheads)

CYTOSKELETAL ELEMENTS

Microfilaments

Microfilaments are actin proteins. They are composed of globular monomers of G-actin that polymerize to form helical filaments of F-actin. Actin polymerization is ATP dependent. The F-actin filaments are 7-nm-diameter filaments that are constantly ongoing assembly and disassembly. F-actin has a distinct polarity. The barbed end (the plus end) is the site of polymerization and the pointed end is the site of depolymerization. Tread milling is the balance in the activity at the 2 ends.

In conjunction with myosin, actin microfilaments provide contractile and motile forces of cells including the formation of a contractile ring that provides a basis for cytokinesis during mitosis and meiosis. Actin filaments are linked to cell membranes at tight junctions and at the zonula adherens, and form the core of microvilli.

Intermediate Filaments

Intermediate filaments are 10-nm-diameter filaments that are usually stable once formed. These filaments provide structural stability to cells. There are 4 types:

- **Type I:** keratins (keratins are found in all epithelial cells)
- **Type II:** intermediate filaments comprising a diverse group
  - Desmin is found in skeletal, cardiac, and GI tract smooth muscle cells.
  - Vimentin is found in most fibroblasts, fibrocytes, endothelial cells, and vascular smooth muscle.
  - Glial fibrillary acidic protein is found in astrocytes and some Schwann cells.
  - Peripherin is found in peripheral nerve axons.
- **Type III:** intermediate filaments forming neurofilaments in neurons
- **Type IV:** 3 types of lamins forming a meshwork rather than individual filaments inside the nuclear envelope of all cells

Clinical Correlate

A first step in the invasion of malignant cells through an epithelium results from a loss of expression of cadherins that weakens the epithelium.

Clinical Correlate

Changes in intermediate filaments are evident in neurons in Alzheimer's disease and in cirrhotic liver diseases.

Microtubules

Microtubules consist of 25-nm-diameter hollow tubes. Like actin, microtubules undergo continuous assembly and disassembly. They provide "tracks" for intracellular transport of vesicles and molecules. Such transport exists in all cells but is particularly important in axons. Transport requires specific ATPase motor molecules; **dynein** drives retrograde transport and **kinesin** drives anterograde transport.

Microtubules are found in **true cilia and flagella**, and utilize dynein to convey motility to these structures. Microtubules form the mitotic spindle during mitosis and meiosis.

Clinical Correlate

Colchicine prevents microtubule polymerization and is used to prevent neutrophil migration in gout. Vinblastine and vincristine are used in cancer therapy because they inhibit the formation of the mitotic spindle.

CELL ADHESION MOLECULES

Cell adhesion molecules are surface molecules that allow cells to adhere to one another or to components of the extracellular matrix. The expression of adhesion molecules on the surface of a given cell may change with time, altering its interaction with adjacent cells or the extracellular matrix.

Cadherin and selectin are adhesion molecules that are **calcium ion-dependent**. The extracellular portion binds to a cadherin dimer on another cell (trans binding). The cytoplasmic portions of cadherins are linked to cytoplasmic actin filaments by the catenin complex of proteins.

Integrins are adhesion molecules that are **calcium-independent**. They are transmembrane surface molecules with extracellular domains that bind to fibronectin and laminin, which are components of extracellular basement membrane. The cytoplasmic portions of integrins bind to actin filaments. Integrins form a portion of hemidesmosomes but are also important in interactions between leukocytes and endothelial cells.

CELL SURFACE SPECIALIZATIONS

Cell Adhesion

A cell must physically interact via cell surface molecules with its external environment, whether it be the extracellular matrix or **basement membrane.** The basement membrane is a sheet-like structure underlying virtually all epithelia, which consists of **basal lamina** (made of type IV collagen, glycoproteins [e.g., laminin], and proteoglycans [e.g., heparin sulfate]), and **reticular lamina** (composed of reticular fibers). Cell junctions anchor cells to each other, seal boundaries between cells, and form channels for direct transport and communication between cells. The 3 types of junctional complex include **anchoring, tight,** and **gap junctions**.

Cell Junctions

Tight junctions (zonula occludens) function as barriers to diffusion and determine cell polarity. They form a series of punctate contacts of adjacent epithelial cells near the apical end or luminal surface of epithelial cells. The major components of tight junctions are occludens (ZO-1,2,3) and claudin proteins. These proteins span between the adjacent cell membranes and their cytoplasmic parts bind to actin microfilaments.

Zonula adherens forms a belt around the entire apicolateral circumference of the cell, immediately below the tight junction of epithelium. Cadherins span between the cell membranes. Like the tight junctions immediately above them, the cytoplasmic parts of cadherins are associated with actin filaments.

Desmosomes (macula adherens) function as anchoring junctions. Desmosomes provide a structural and mechanical link between cells. Cadherins span between the cell membranes of desmosomes and internally desmosomes are anchored to intermediate filaments in large bundles called tonofilaments.

Hemidesmosomes adhere epithelial cells to the basement membrane. The basement membrane is a structure that consists of the basal membrane of a cell

Clinical Correlate

Pemphigus Vulgaris

- Autoantibodies against desmosomal proteins in skin cells
- Painful flaccid bullae (blisters) in oropharynx and skin that rupture easily
- Postinflammatory hyperpigmentation
- Treatment: corticosteroids

Bullous Pemphigoid

- Autoantibodies against basement-membrane hemidesmosomal proteins
- Widespread blistering with pruritus
- Less severe than pemphigus vulgaris
- Rarely affects oral mucosa
- Can be drug-induced (e.g., middle-aged or elderly patient on multiple medications)
- Treatment: corticosteroids

and 2 underlying extracellular components, the basal lamina and the reticular lamina. The basal lamina is a thin felt-like extracellular layer composed of predominantly of type IV collagen associated with laminin, proteoglycans, and fibronectin that are secreted by epithelial cells. Fibronectin binds to integrins on the cell membrane, and fibronectin and laminin in turn bind to collagen in the basal lamina. Internally, like a desmosome, the hemidesmosomes are linked to intermediate filaments. Below the basal lamina is the reticular lamina, composed of reticular fibers.

Through the binding of extracellular components of hemidesmosomes to integrins, and thus to fibronectin and laminin, the cell is attached to the basement membrane and therefore to the extracellular matrix components outside the basement membrane. These interactions between the cell cytoplasm and the extracellular matrix have implications for permeability, cell motility during embryogenesis, and cell invasion by malignant neoplasms.

Gap junctions (communicating junctions) function in cell-to-cell communication between the cytoplasm of adjacent cells by providing a passageway for ions such as calcium and small molecules such as cyclic adenosine monophosphate (cAMP). The transcellular channels that make up a gap junction consist of connexons, which are hollow channels spanning the plasma membrane. Each connexon consists of 6 connexin molecules. Unlike other intercellular junctions, gap junctions are not associated with any cytoskeletal filament.

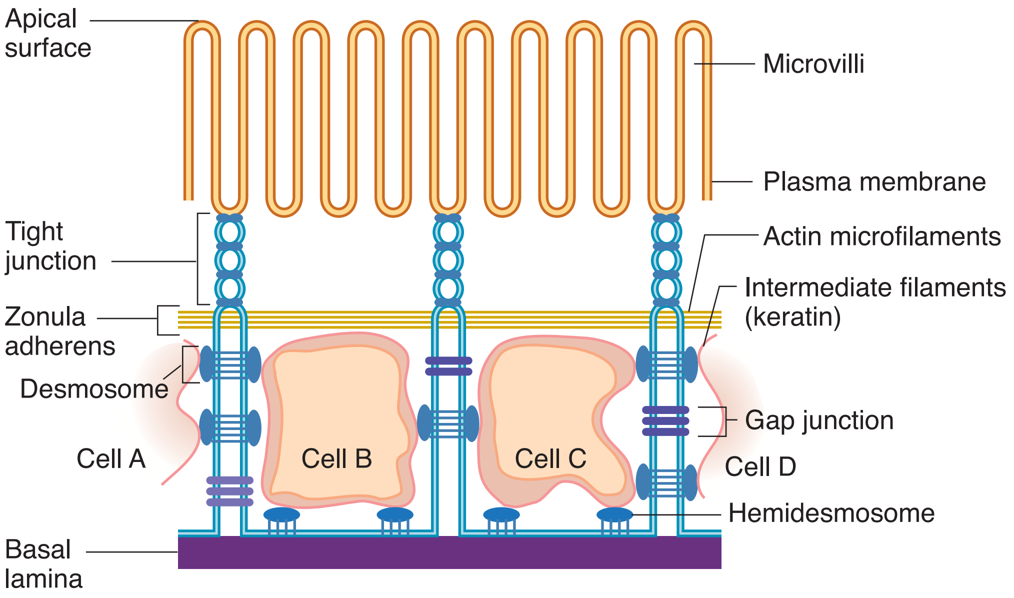

Figure I-3-9. Junctions

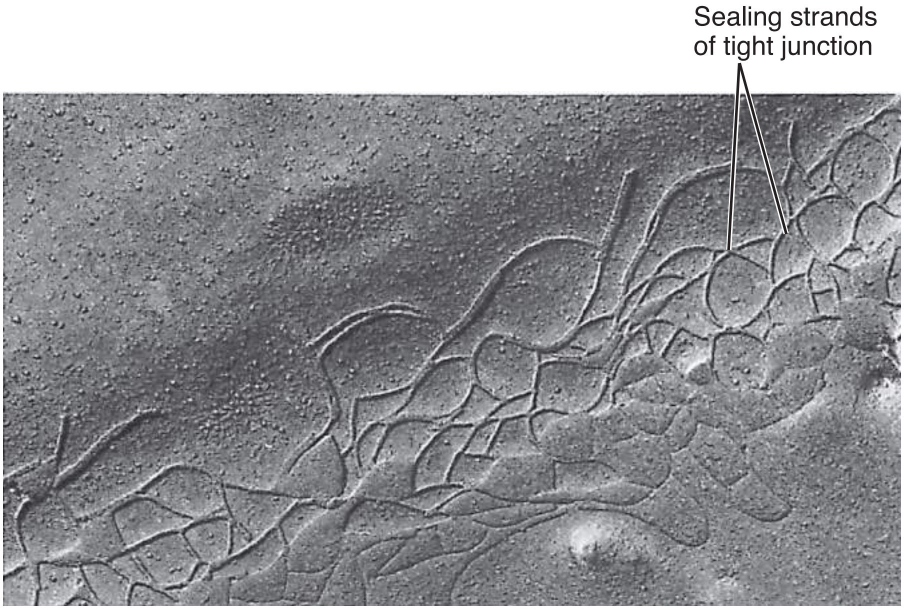

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Figure I-3-10. Freeze-fracture of Tight Junction

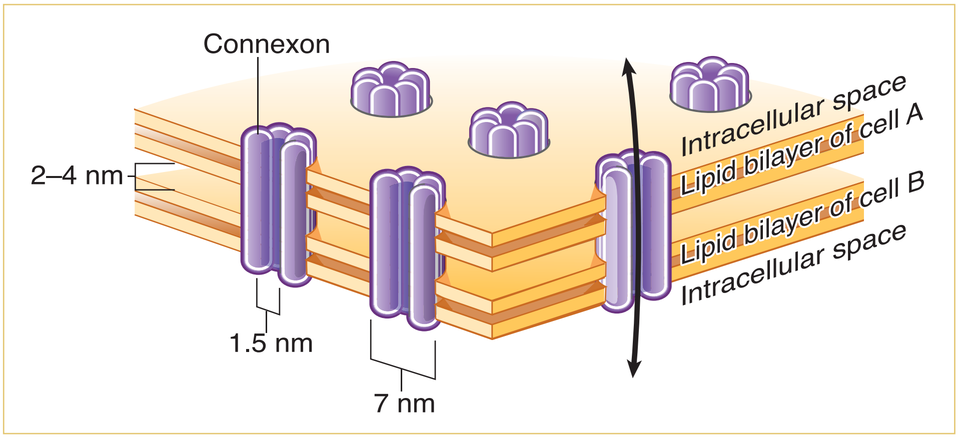


Figure I-3-11. Gap Junction

Microvilli

Microvilli contain a core of actin microfilaments and function to increase the absorptive surface area of an epithelial cell. They are found in columnar epithelial cells of the small and large intestine, cells of the proximal tubule of the kidney and on columnar epithelial respiratory cells.

Stereocilia are long, branched microvilli that are found in the male reproductive tract (e.g., epididymis). Short stereocilia cap all sensory cells in the inner ear.

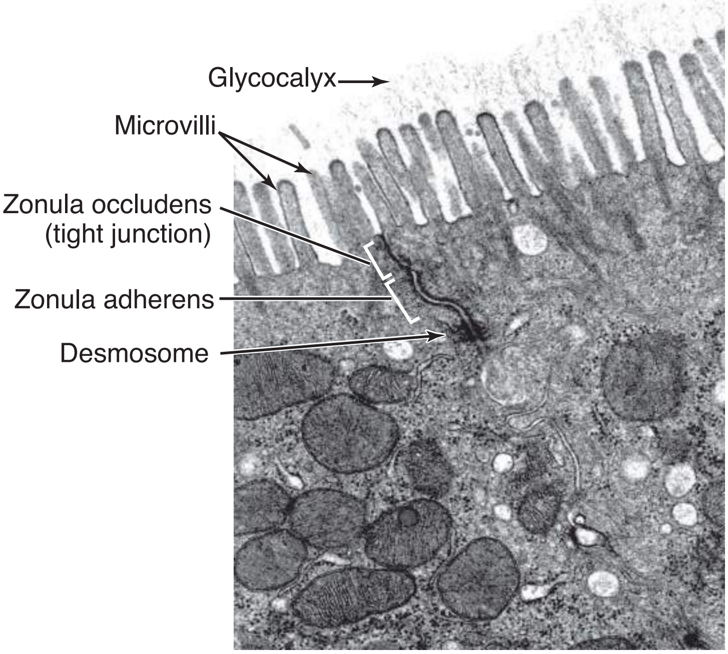
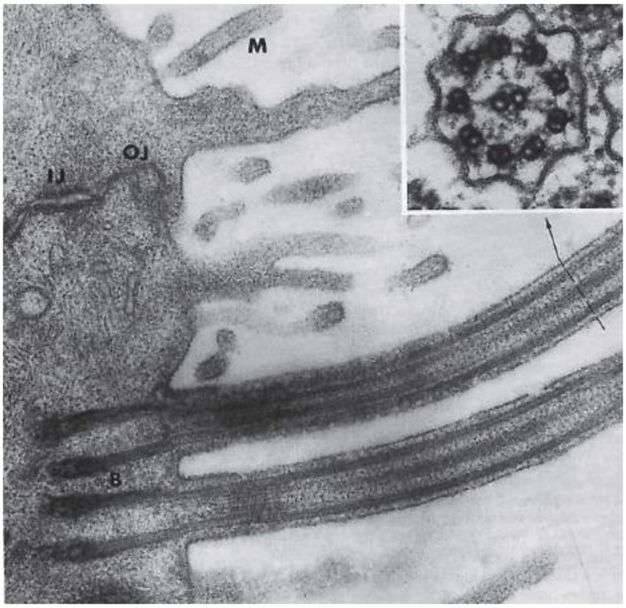


Figure I-3-12. Apical Cell Surface/Cell Junctions

Cilia

Cilia contain 9 peripheral pairs of microtubules and 2 central microtubules. The microtubules convey motility to cilia through the ATPase dynein. Cilia bend and beat on the cell surface of pseudostratified ciliated columnar respiratory epithelial cells to propel overlying mucous. They also form the core of the flagella, the motile tail of sperm cells.



B: basal body
IJ: intermediate junction
M: microvillus
OJ: occluding junction

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Figure I-3-13. Cilia

Clinical Correlate

Kartagener syndrome is characterized by immotile spermatozoa and infertility. It is due to an absence of dynein that is required for flagellar motility. It is usually associated with chronic respiratory infections because of similar defects in cilia of respiratory epithelium.

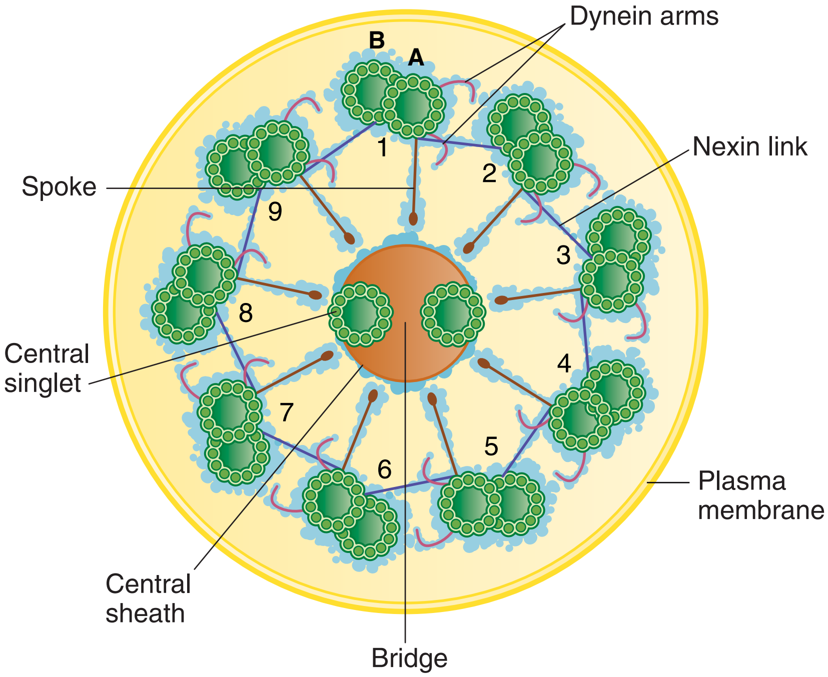

Figure I-3-14. Structure of the Axoneme of a Cilium

PART II

Gross Anatomy

Back and Autonomic Nervous System 1

Learning Objectives

- ❑ Solve problems concerning vertebral column
- ❑ Demonstrate understanding of spinal meninges
- ❑ Use knowledge of spinal nerves
- ❑ Use knowledge of autonomic nervous system

VERTEBRAL COLUMN

Embryology

During week 4, **sclerotome** cells of the somites (mesoderm) migrate medially to surround the spinal cord and notochord. After proliferation of the caudal portion of the sclerotomes, the vertebrae are formed, each consisting of the caudal part of one sclerotome and the cephalic part of the next.

Vertebrae

The vertebral column is the central component of the axial skeleton which functions in muscle attachments, movements, and articulations of the head and trunk.

- The vertebrae provide a flexible support system that transfers the weight of the body to the lower limbs and also provides protection for the spinal cord.
- The vertebral column is composed of 32–33 vertebrae (7 cervical, 12 thoracic, 5 lumbar, and the fused 5 sacral, and 3–4 coccygeal), intervertebral disks, synovial articulations (zygapophyseal joints) and ligaments.

~33 vertebrae

31 spinal nerves

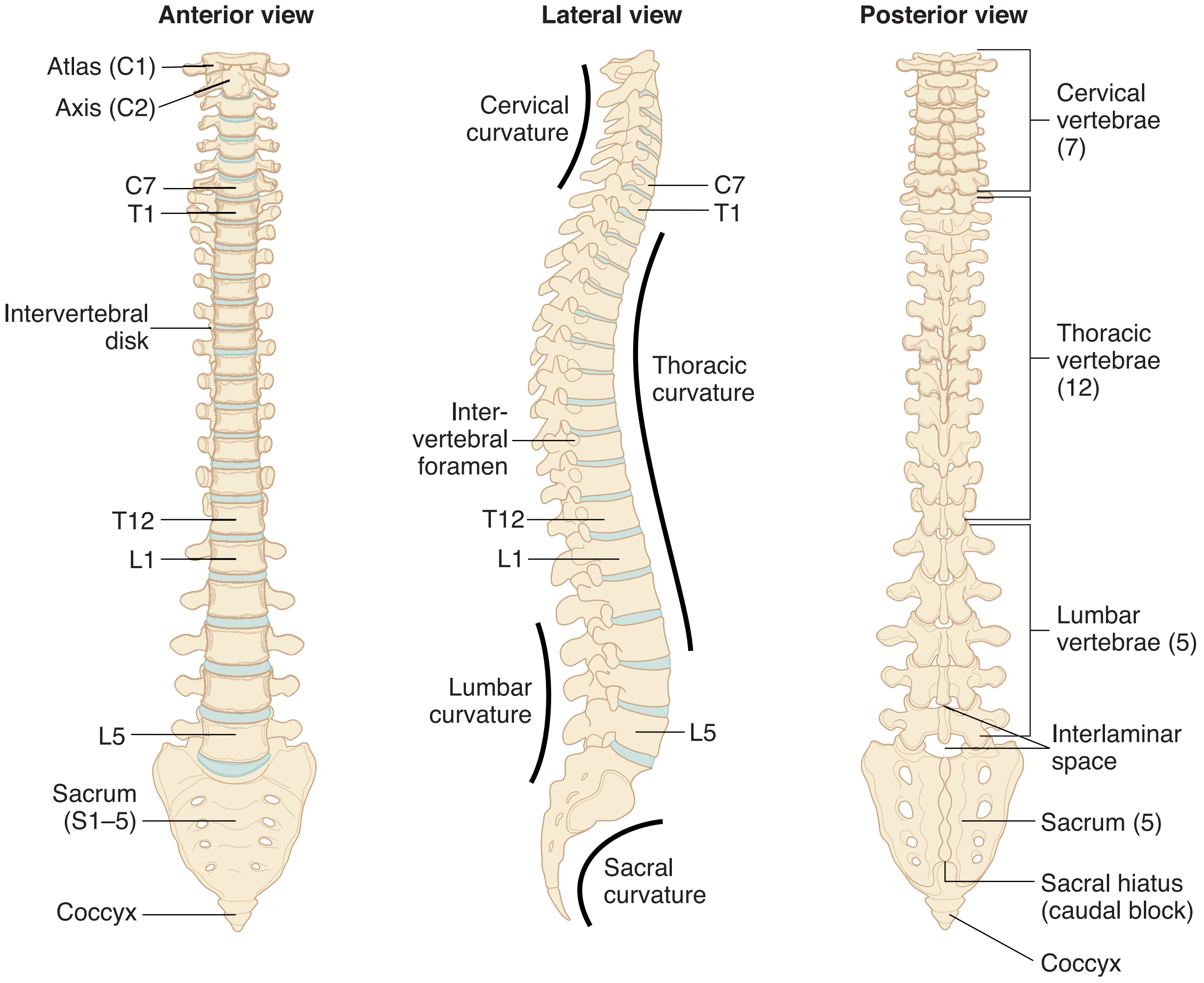

Figure II-1-1. Vertebral Column

A **typical vertebra** consists of an anterior **body** and a posterior **vertebral arch** consisting of 2 **pedicles** and 2 **laminae**. The vertebral arch encloses the **vertebral (foramen) canal** that houses the spinal cord. **Vertebral notches** of adjacent pedicles form **intervertebral foramina** that provide for the exit of the spinal nerves. The dorsal projecting **spines** and the lateral projecting **transverse processes** provide attachment sites for muscles and ligaments.

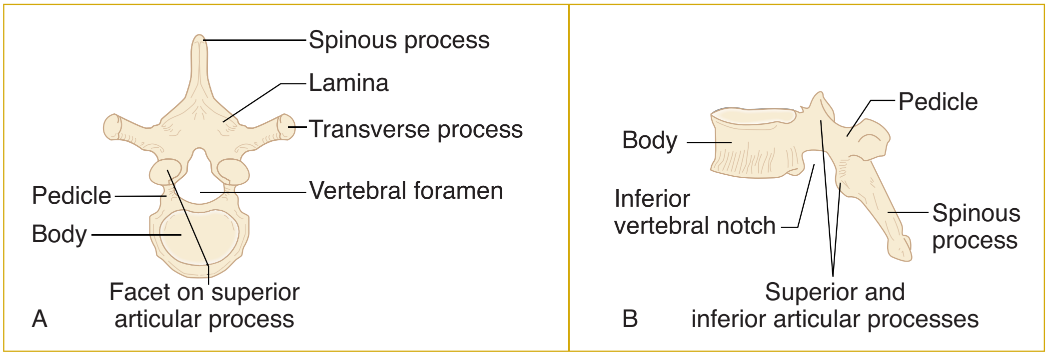

Figure II-1-2. Typical Vertebra

Intervertebral Disks

The **intervertebral disks** contribute to about 25% of the length of the vertebral column. They form the cartilaginous joints between the vertebral bodies and provide limited movements between the individual vertebrae.

- Each intervertebral disk is numbered by the vertebral body **above** the disk.
- Each intervertebral disk is composed of the following:
  - **Anulus fibrosus** consists of the outer concentric rings of fibrocartilage and fibrous connective tissue. The anuli connect the adjacent bodies and provide limited movement between the individual vertebrae.
  - **Nucleus pulposus** is an inner soft, elastic, compressible material that functions as a shock absorber for external forces placed on the vertebral column. The nucleus pulposus is the postnatal remnant of the **notochord.**

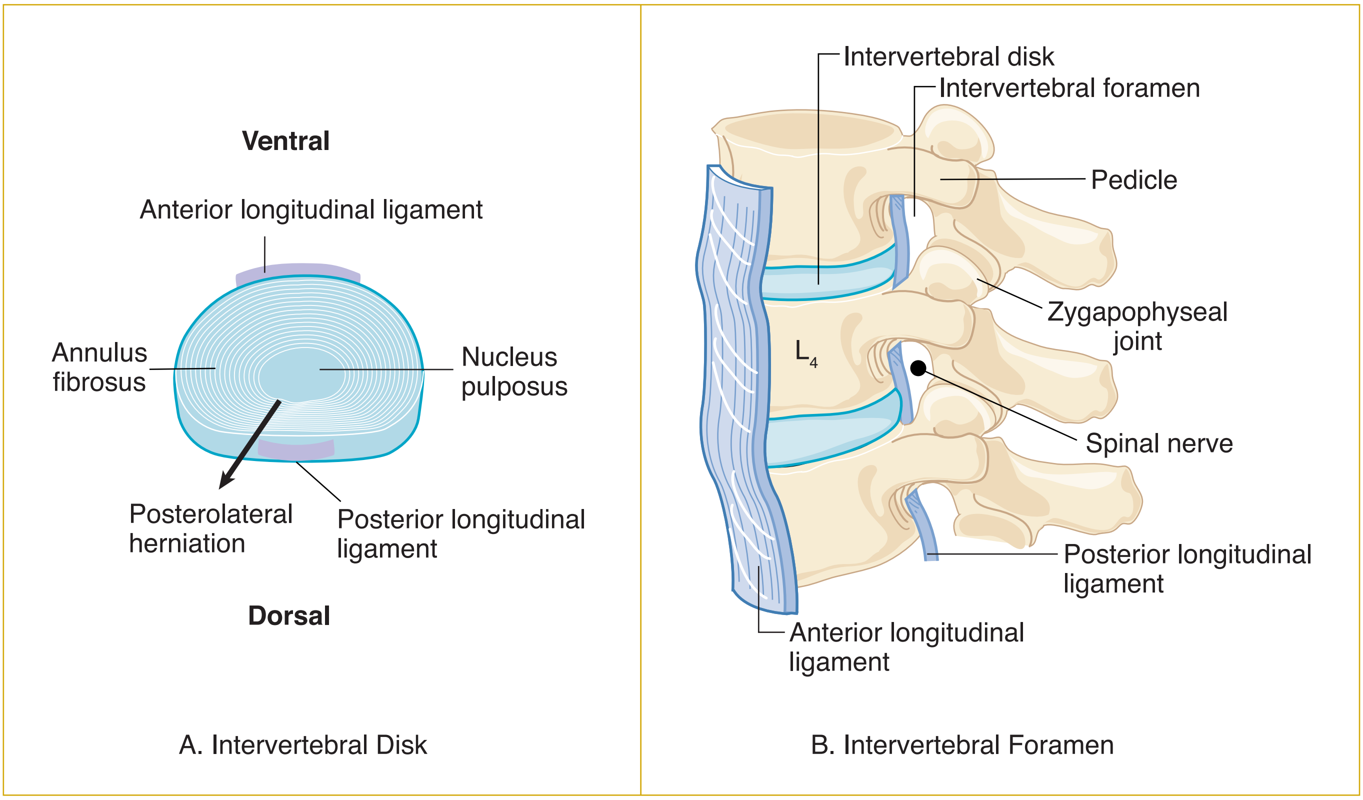

Figure II-1-3. Intervertebral Disks

Clinical Correlate

The herniation of a nucleus pulposus is most commonly in a **posterolateral** direction due to the strength and position of the posterior longitudinal ligament (Figure II-1-3-A).

Ligaments of the Vertebral Column

The vertebral bodies are strongly supported by 2 longitudinal ligaments. Both ligaments are firmly attached to the intervertebral disks and to the bodies of the vertebrae.

- **Anterior longitudinal ligament** forms a broad band of fibers that connects the anterior surfaces of the bodies of the vertebrae between the cervical and sacral regions. It prevents **hyperextension** of the vertebrae and is often involved in “whiplash” accidents.
- **Posterior longitudinal ligament** connects the posterior surfaces of the vertebral bodies and is located in the vertebral canal. It limits **flexion** of the vertebral column. This ligament causes the herniation of a disk to be positioned **posterolaterally**.

Herniated Disk

The **nucleus pulposus** may herniate through the **anulus fibrosus**. The herniated nucleus pulposus may compress the spinal nerve roots, resulting in pain along the involved spinal nerve (sciatica).

- Herniation usually occurs in the lower lumbar (L4/L5 or L5/S1) or lower cervical (C5/C6 or C6/C7) parts of the vertebral column.
- The herniated disk will usually compress the spinal nerve roots **one number below** the involved disk (e.g., the herniation of the L4 disk will compress the L5 roots, or herniation of the C7 disk will compress the C8 nerve roots).

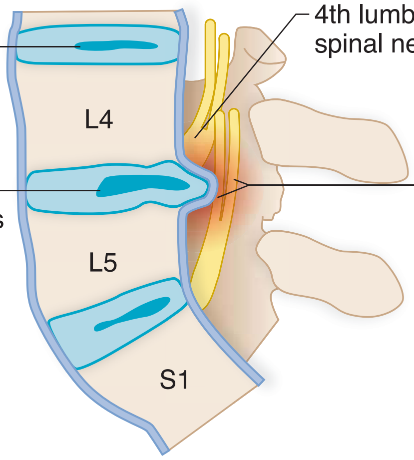

Figure II-1-4. Herniated Intervertebral Disk

Intervertebral Foramen

The intervertebral foramina are formed by successive intervertebral notches and provide for the passage of the **spinal nerve**. The boundaries of the foramina are:

- **Anterior: bodies** of the vertebrae and **intervertebral disks**
- **Posterior: zygapophyseal joint** and **articular processes**
- **Superior and inferior: pedicles** of the vertebrae

SPINAL MENINGES

The spinal cord is protected and covered by 3 connective tissue layers within the vertebral canal: the dura mater, arachnoid, and pia mater.

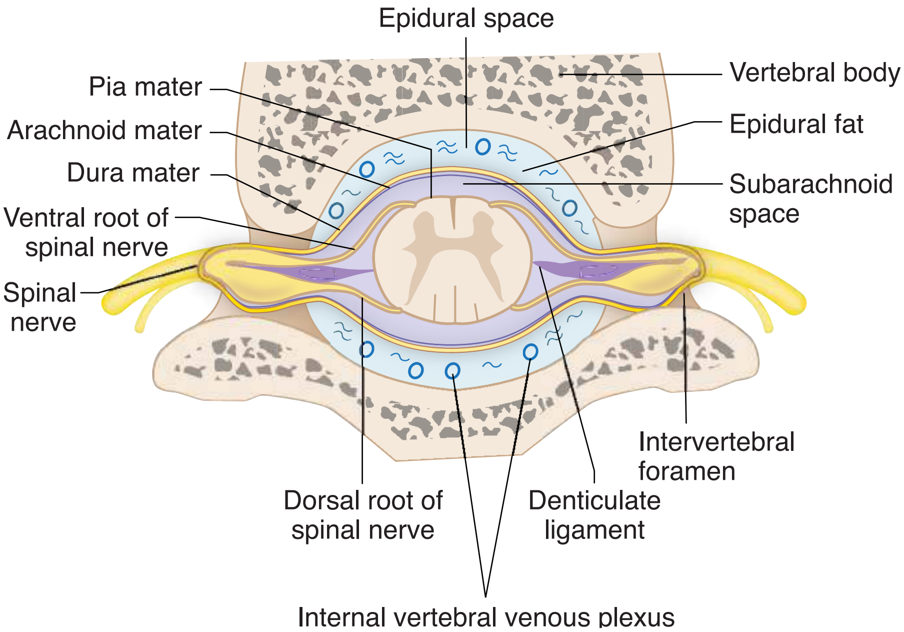

Figure II-1-5. Cross-Section of Vertebral Canal

Dura Mater

The **dura mater** is a tough, cylindrical covering of connective tissue forming a **dural sac** which envelops the entire spinal cord and cauda equina.

- The dura mater and dural sac terminate inferiorly at the **second sacral vertebra level**.
- Superiorly, the dura mater continues through the foramen magnum and is continuous with the meningeal layer of the cranial dura.

Arachnoid

The **arachnoid** is a delicate membrane which completely lines the inner surface of the dura mater and dural sac. It continues inferiorly and terminates at the **second sacral vertebra**.

Pia Mater

The **pia mater** is tightly attached to the surface of the spinal cord and provides a delicate covering of the cord.

- The spinal cord, with its covering of pia mater, terminates at the **L1 or L2 vertebral levels** in the adult.
- There are 2 specializations of the pia mater that are attached to the spinal cord:
  - The **denticulate ligaments** are bilateral thickenings of pia mater that run continuously on the lateral sides of the midpoint of the cord. They separate the ventral and dorsal roots of the spinal nerves and anchor to the dura mater.

– The **filum terminale** is a continuation of the pia mater distal to the lower end of the spinal cord. The filum terminale is part of the **cauda equina** which is composed of ventral and dorsal roots of lumbar and sacral nerves that extend below the inferior limit of the spinal cord.

There are 2 spaces related to the meninges. The **epidural space** is located between the inner walls of the vertebral canal and the dura mater. It contains fat and the **internal vertebral venous plexus.** The venous plexus runs the entire length of the epidural space and continues superiorly through the foramen magnum to connect with dural venous sinuses in the cranial cavity.

The **subarachnoid space** is a pressurized space located between the arachnoid and pia mater layers. It contains **cerebrospinal fluid (CSF)**, which bathes the spinal cord and spinal nerve roots within the dural sac, and terminates at the **second sacral vertebral level**.

There are 2 important vertebral levels. The **L1** or **L2 vertebrae** is the inferior limit of the **spinal cord** in adults (**conus medullaris**). **S2** vertebra is the inferior limit of the **dural sac** and the **subarachnoid** space (cerebrospinal fluid).

Clinical Correlate

The internal vertebral venous plexus is valveless and connects with veins of the pelvis, abdomen, and thorax. It provides a route of metastasis of cancer cells to the vertebral column and the cranial cavity.

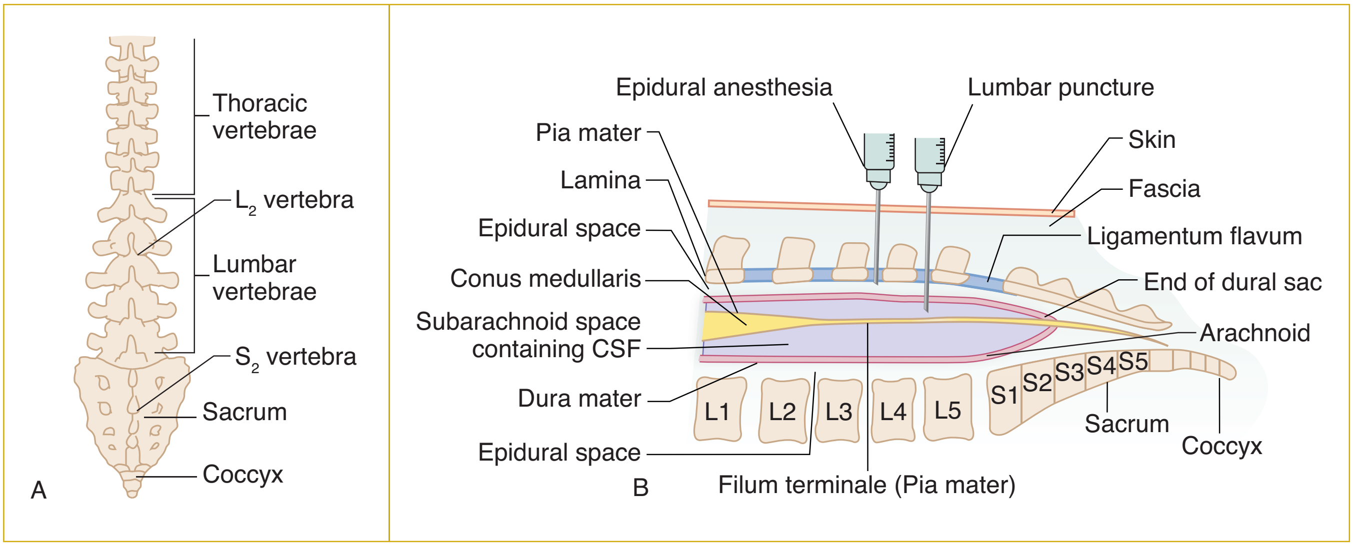

Figure II-1-6. Important Vertebral Levels

SPINAL NERVES

There are 31 pairs of **spinal nerves** attached to each segment of the spinal cord: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal. The spinal nerves with the cranial nerves form part of the **peripheral nervous system**.

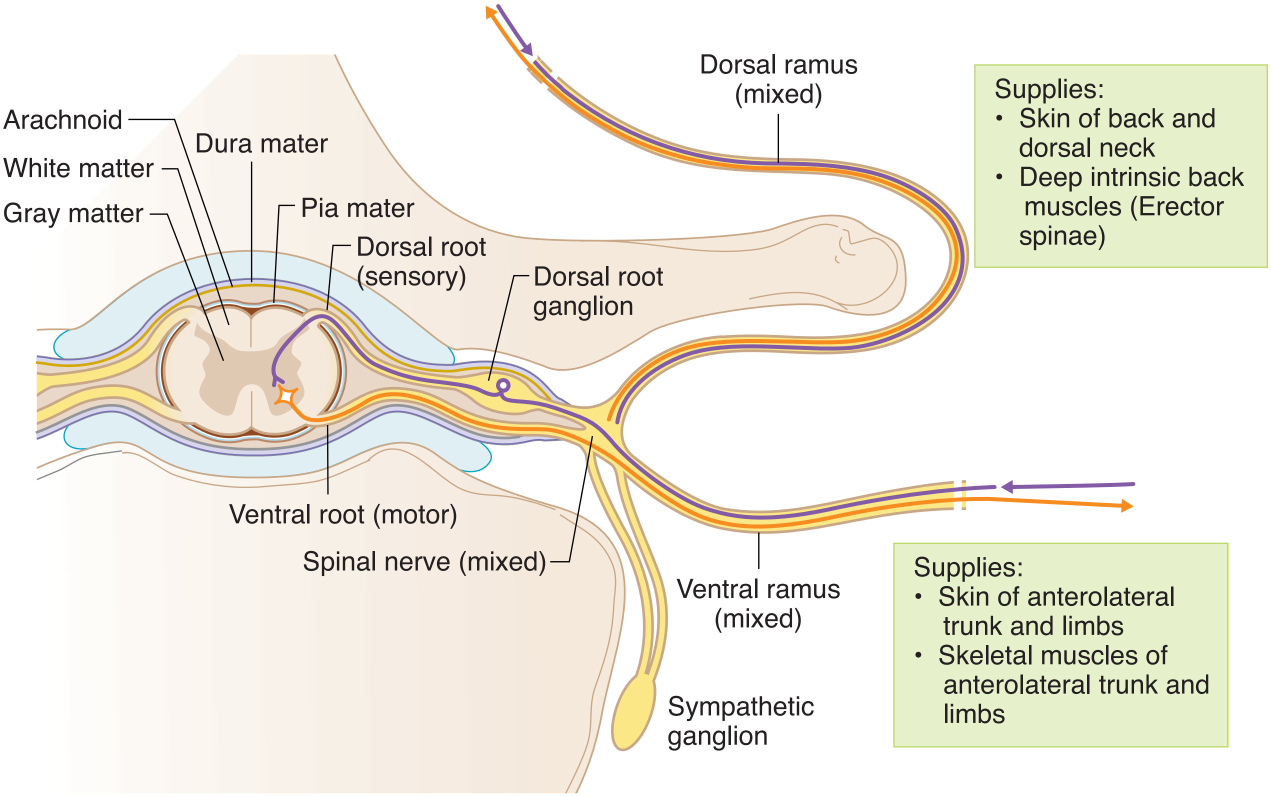

Figure II-1-7. Cross Section of Spinal Cord and Parts of Spinal Nerve

Each spinal nerve is formed by the following components:

- **Dorsal root** carries **sensory** fibers from the periphery into the dorsal aspect of the spinal cord; on each dorsal root there is a **dorsal root ganglion** (sensory) containing the pseudounipolar cell bodies of the nerve fibers that are found in the dorsal root
- **Ventral root** arises from the ventral aspect of the spinal cord and carries axons of **motor** neurons from the spinal cord to the periphery; the cell bodies of the axons in the ventral root are located in the ventral or lateral horns of the spinal cord gray matter
- **Spinal nerve** is formed by the union of the ventral and dorsal roots; it exits the vertebral column by passing through the intervertebral foramen
- **Dorsal rami** innervate the skin of the dorsal surface of the back, neck, zygapophyseal joints, and intrinsic skeletal muscles of the deep back
- **Ventral rami** innervate the skin of the anterolateral trunk and limbs, and the skeletal muscles of the anterolateral trunk and limbs (ventral rami form the brachial and lumbosacral plexuses)

The spinal nerves exit the vertebral column by a **specific relationship to the vertebrae**. The cervical nerves C1–C7 exit the intervertebral foramina **superior** to the pedicles of the same-numbered vertebrae. The C8 nerve exits the intervertebral foramen **inferior** to the C7 pedicle. This is the **transition point**. All nerves beginning with T1 and below will exit the intervertebral foramina **inferior** to the pedicle of the same-numbered vertebrae.

Lumbar Puncture

A **lumbar puncture** is used to inject anesthetic material in the epidural space or to withdraw CSF from the subarachnoid space.

- A spinal tap is typically performed at the **L4-L5 interspace**.
- A horizontal line drawn at the **top of the iliac crest** marks the level of the L4 vertebra.
- When a lumbar puncture is performed in the midline, the needle passes through the **interlaminar space** of the vertebral column found between the laminae of the lumbar vertebrae.
- The interlaminar spaces are covered by the highly elastic **ligamenta flava**.

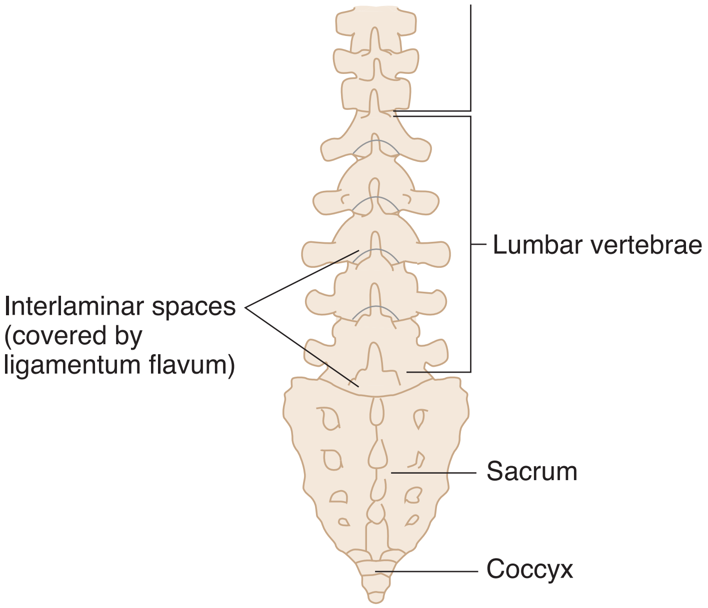

Figure II-1-8. Interlaminar Spaces

Clinical Correlate

During a lumbar puncture, a needle is passed through the interlaminar space while the vertebral column is flexed. The needle passes through the following layers:

- Skin
- Superficial fascia
- Deep fascia
- Supraspinous ligament
- Interspinous ligament
- Interlaminar space
- Epidural space
- Dura
- Arachnoid
- Subarachnoid space

AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) is concerned with the motor innervation of smooth muscle, cardiac muscle, and glands of the body. Anatomically and functionally, it is composed of 2 motor divisions: **sympathetic** and **parasympathetic**. In both divisions, 2 neurons form an autonomic pathway.

- **Preganglionic neurons have** their neuronal cell bodies in the **CNS** (formed by neuroectoderm); their axons exit in cranial and spinal nerves.
- **Postganglionic neurons have** cell bodies in **autonomic ganglia** in the **peripheral nervous system** (PNS) (formed by neural crest cells)

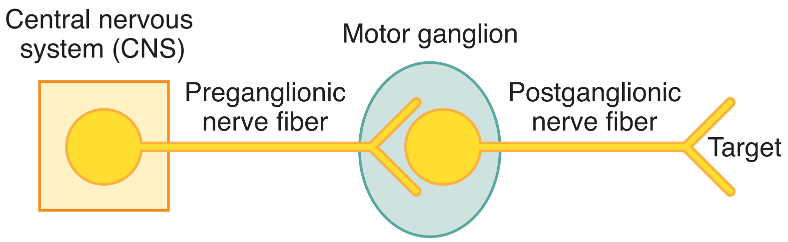

Figure II-1-9. Autonomic Nervous System

Sympathetic Nervous System

The **preganglionic cell bodies** of the sympathetic nervous system are found in the **lateral horn** gray matter of spinal cord segments **T1–L2** (14 segments).

The **postganglionic cell bodies** of the sympathetic system are found in one of 2 types of motor ganglia in the PNS:

- **Chain or paravertebral**
- **Collateral or prevertebral** (found only in abdomen or pelvis)

Table II-1-1. Sympathetic = Thoracolumbar Outflow

Origin (Preganglionic)	Site of Synapse (Postganglionic)	Innervation (Target)
Spinal cord levels T1–L2	Sympathetic chain ganglia (paravertebral ganglia)	Smooth muscle, cardiac muscle and glands of **body wall** and **limbs** (T1–L2), **head** (T1–2) and **thoracic viscera** (T1–5).
Thoracic splanchnic nerves T5–T12	Prevertebral ganglia (collateral) (e.g., celiac, aorticorenal, superior mesenteric ganglia)	Smooth muscle and glands of the **foregut** and **midgut**
Lumbar splanchnic nerves L1–L2	Prevertebral ganglia (collateral) (e.g., inferior mesenteric and pelvic ganglia)	Smooth muscle and glands of the **pelvic viscera** and **hindgut**

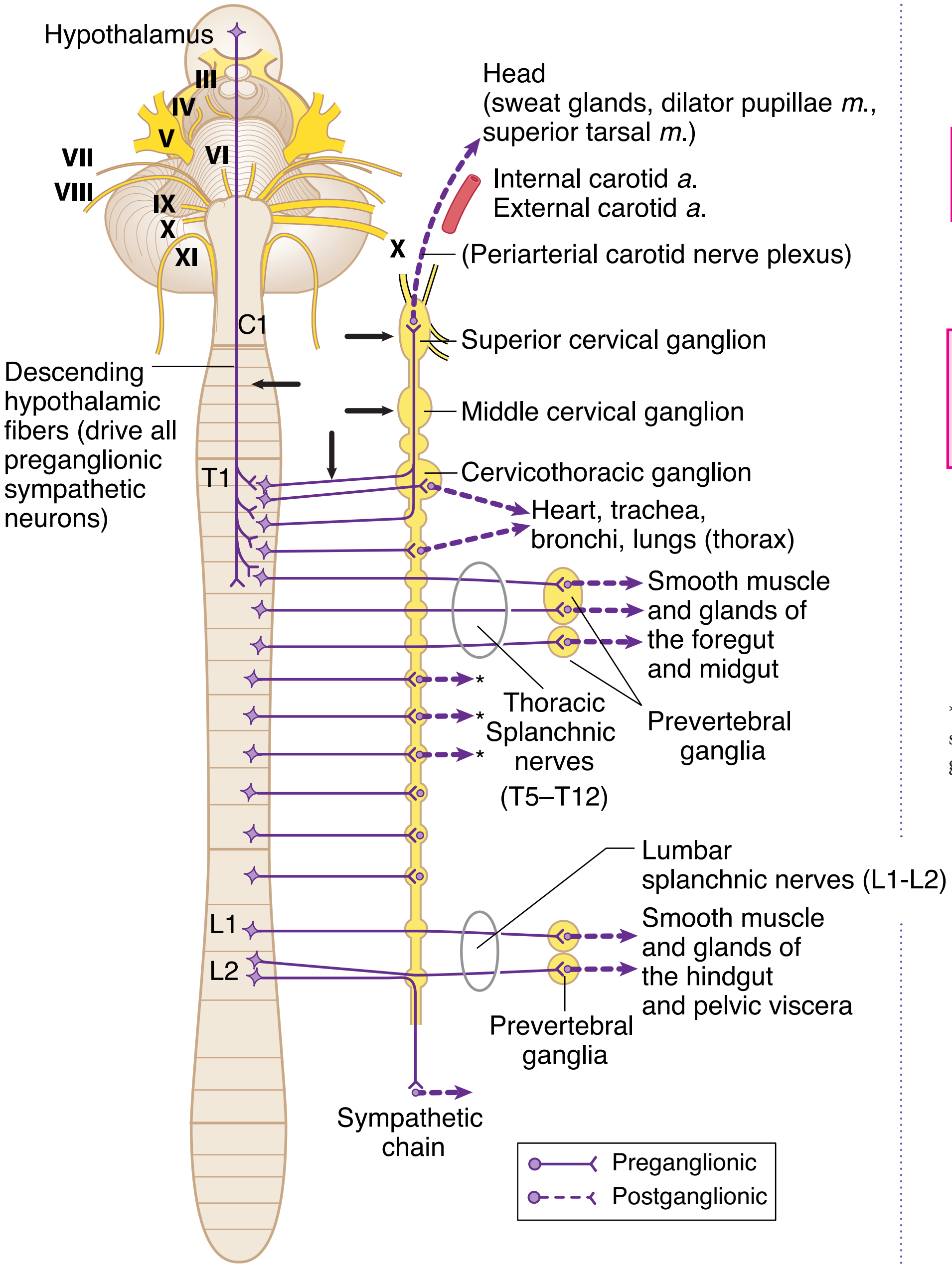

Figure II-1-10. Overview of Sympathetic Outflow

m: muscle
a: artery

Lesions at arrows result in ipsilateral Horner syndrome (ptosis, miosis, and anhydrosis).

*Gray rami carry postganglionic sympathetic axons from the sympathetic ganglion to the spinal nerve.

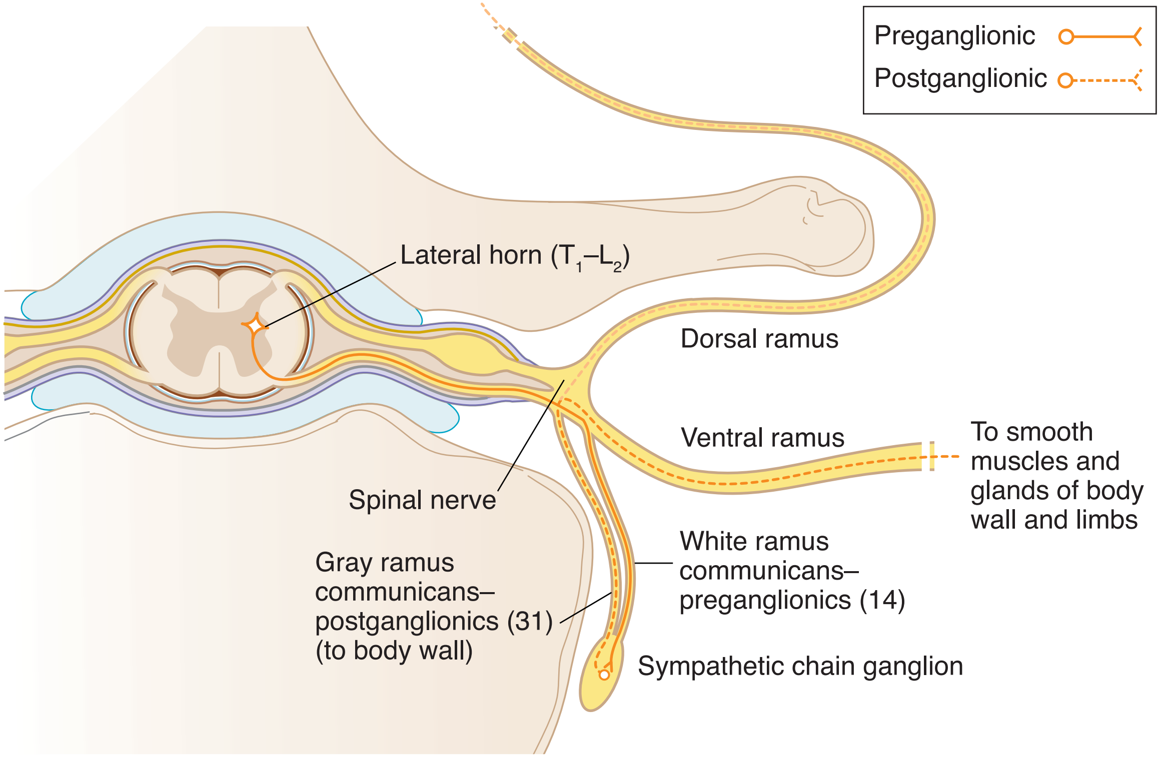

Figure II-1-11. Cross Section of Spinal Cord Showing Sympathetic Outflow

Note

White rami are preganglionic sympathetics that all enter the sympathetic trunk of ganglia. They may synapse with ganglion at point of entry or go up or down and synapse above or below point of entry. If a white ramus does not synapse, it passes through ganglion and becomes a root of a thoracic or lumbar splanchnic nerve.

Parasympathetic Nervous System

The **preganglionic cell bodies** of the parasympathetic nervous system are found in the **CNS** in one of 2 places:

- Gray matter of brain stem associated with **cranial nerves III, VII, IX, and X,** or
- Spinal cord gray in **sacral segments S2 3, and 4 (pelvic splanchnics)**

The **postganglionic cell bodies** of the parasympathetic nervous system are found in **terminal ganglia** in the PNS that are usually located near the organ innervated or in the wall of the organ.

Table II-1-2. Parasympathetic = Craniosacral Outflow

Origin (Preganglionic)	Site of Synapse (Postganglionic)	Innervation (Target)
Cranial nerves III, VII, IX	4 cranial ganglia	Glands and smooth muscle of the **head**
Cranial nerve X	Terminal ganglia (in or near the walls of viscera)	Viscera of the neck, **thorax, foregut,** and **midgut**
Pelvic splanchnic nerves S 2, 3, 4	Terminal ganglia (in or near the walls of viscera)	**Hindgut** and **pelvic viscera** (including the bladder, rectum, and erectile tissue)

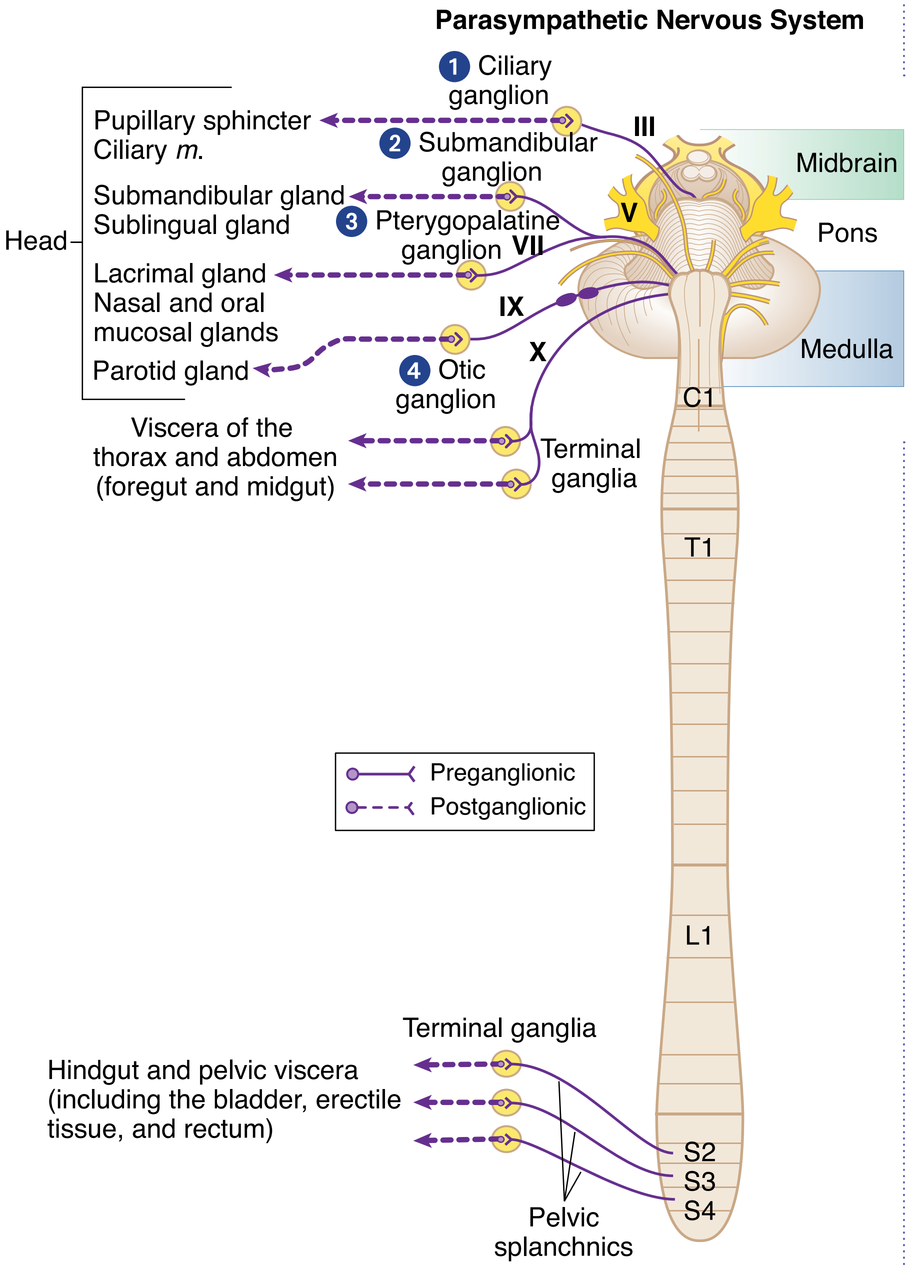

Figure II-1-12. Overview of Parasympathetic Outflow

Thorax 2

Learning Objectives

- ❑ Solve problems concerning the chest wall
- ❑ Use knowledge of embryology of lower respiratory system
- ❑ Use knowledge of pleura and pleural cavity
- ❑ Interpret scenarios on respiratory histology
- ❑ Use knowledge of alveolar ducts, alveolar sacs, and the alveoli
- ❑ Answer questions about embryology of the heart
- ❑ Solve problems concerning the mediastinum
- ❑ Interpret scenarios on heart histology
- ❑ Solve problems concerning the diaphragm

CHEST WALL

Breast

The breast (mammary gland) is a subcutaneous glandular organ of the superficial pectoral region. It is a modified sweat gland, specialized in women for the production and secretion of milk. A variable amount of fat surrounds the glandular tissue and duct system and is responsible for the shape and size of the female breast.

- **Cooper ligaments** are suspensory ligaments that attach the mammary gland to the skin and run from the skin to the deep fascia.
- There is an extensive **blood supply** to the mammary tissues. The 2 prominent blood supplies are:
  - **Internal thoracic artery (internal mammary)**, a branch of the subclavian artery which supplies medial aspect of the gland
  - **Lateral thoracic artery**, a branch of the axillary artery which contributes to the blood supply to lateral part of the gland; lateral aspect of the chest wall, the lateral thoracic artery courses with the **long thoracic nerve**, superficial to serratus anterior muscle

Clinical Correlate

The presence of a tumor within the breast can distort Cooper ligaments, which results in dimpling of the skin (orange-peel appearance).

Clinical Correlate

During a radical mastectomy, **the long thoracic nerve** (serratus anterior muscle) may be lesioned during ligation of the lateral thoracic artery.
A few weeks after surgery, the patient may present with a **winged scapula** and weakness in abduction of the arm above 90°. The **thoracodorsal nerve** supplying the latissimus dorsi muscle may also be damaged during mastectomy, resulting in weakness in extension and medial rotation of the arm.

- The **lymphatic drainage** of the breast is critical due to its important role in metastasis of breast cancer. The lymphatic drainage of the breast follows 2 primary routes:
  - Laterally, most of the lymphatic flow (75%) drains from the nipple and the superior, lateral, and inferior quadrants of the breast to the **axillary nodes**, initially to the **pectoral group**.
  - From the medial quadrant, most lymph drains to the **parasternal nodes**, which accompany the internal thoracic vessels. It is also through this medial route that cancer can spread to the **opposite breast**.

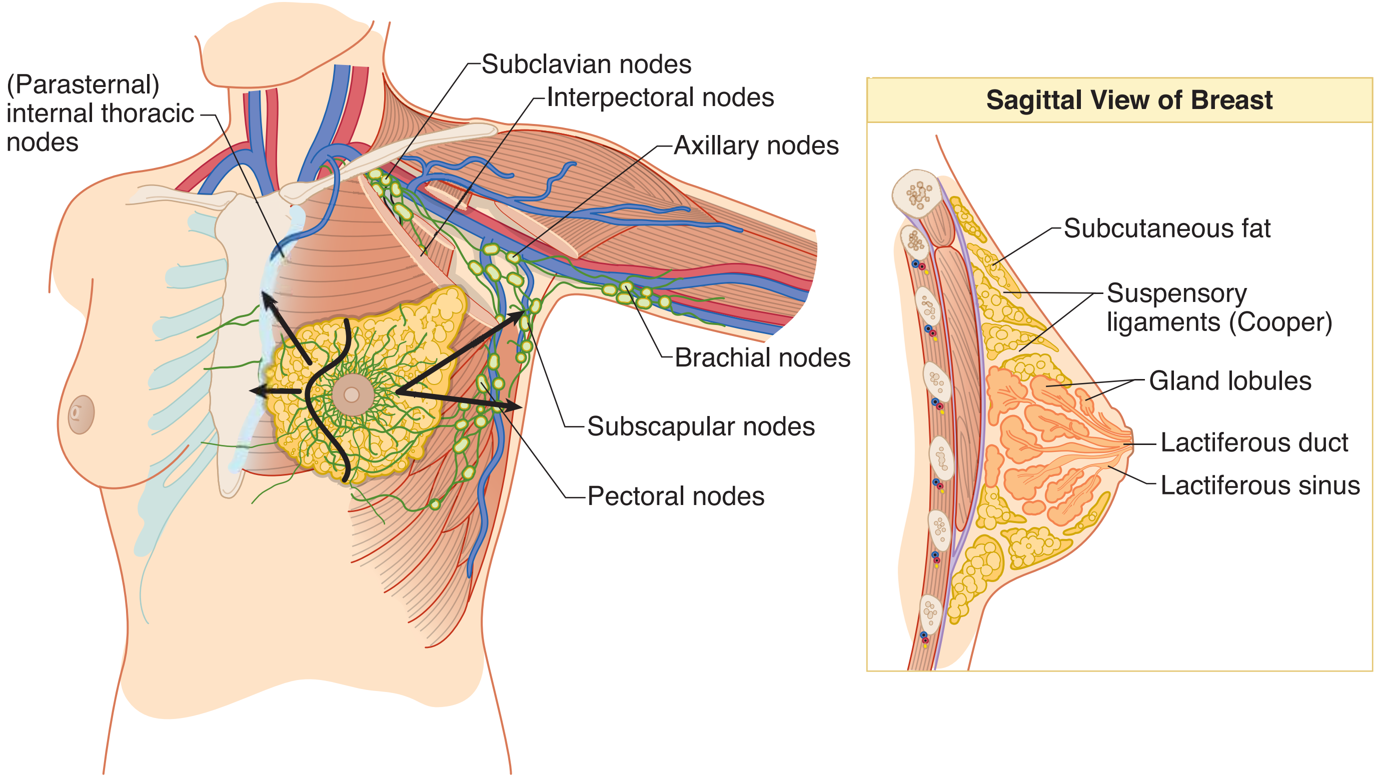

Figure II-2-1. Breast

EMBRYOLOGY OF LOWER RESPIRATORY SYSTEM

During week 4 of development, the lower respiratory system (trachea, bronchi, and lungs) begins to develop as a single **respiratory (laryngotracheal) diverticulum** of endoderm from the ventral wall of the **foregut**. The respiratory epithelium develops from **endoderm** while the muscles, connective tissues, and cartilages develop from **mesoderm**.

- The respiratory diverticulum enlarges distally to form the **lung bud.**
- The diverticulum and lung bud then bifurcate into the 2 **bronchial buds**, which then undergo a series of divisions to form the major part of the bronchial tree (main, secondary, and tertiary bronchi) by month 6.
- The tertiary segmental bronchi are related to the bronchopulmonary segments of the lungs.

- To separate the initial communication with foregut, the **tracheoesophageal septum** forms to separate the esophagus from the trachea.
- A critical time in lung development is the **25–28th** weeks. By this time, the Type I and II pneumocytes are present and gas exchange and surfactant production are possible. Premature fetuses born during this time can survive with intensive care. The amount of surfactant production is critical.

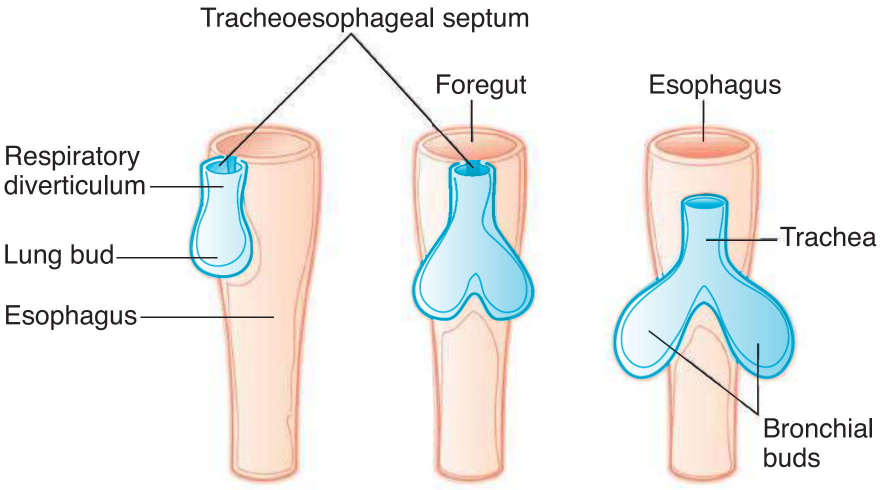

Figure II-2-2. Development of the Lower Respiratory System

A **tracheoesophageal fistula** is an abnormal communication between the trachea and esophagus caused by a malformation of the tracheoesophageal septum. It is generally associated with the following:

- **Esophageal atresia** and **polyhydramnios** (increased volume of amniotic fluid)
- Regurgitation of milk
- Gagging and cyanosis after feeding
- Abdominal distention after crying
- **Reflux** of gastric contents into lungs causing pneumonitis

The fistula is most commonly (90% of cases) located between the esophagus and **distal third of the trachea**.

Clinical Correlate

Pulmonary hypoplasia occurs when lung development is stunted. This condition has 2 congenital causes: (1) **congenital diaphragmatic hernia** (a herniation of abdominal contents into the thorax, which affects the development of the left lung), or (2) **bilateral renal agenesis** (this causes **oligohydramnios**, which increases the pressure on the fetal thorax and Potter's sequence). One of the features of Potter's sequence is bilateral pulmonary hypoplasia.

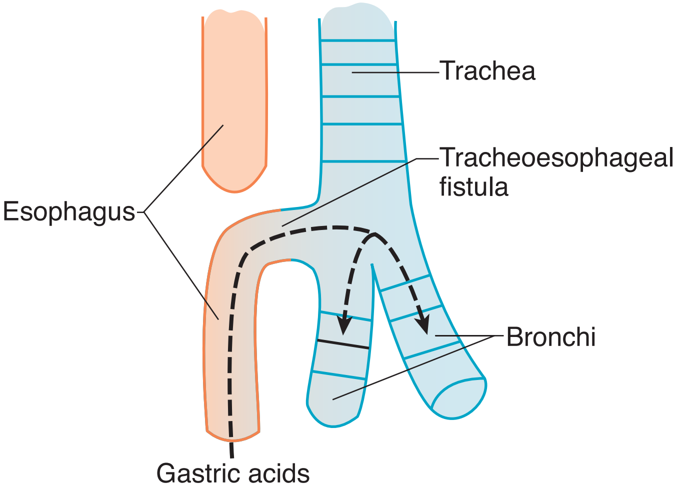

Figure II-2-3. Tracheoesophageal Fistula (Most Common Type)

ADULT THORACIC CAVITY

The **thoracic cavity** is kidney-shaped on cross section and is bounded anterolaterally by the bony thorax (sternum, ribs, and intercostal spaces) and posteriorly by the thoracic vertebrae. Superiorly, the thoracic cavity communicates through the **thoracic inlet** with the base of the neck. (Note, however, that clinically this region is usually called the thoracic outlet.) Inferiorly, the **thoracic outlet** is closed by the diaphragm which separates the thoracic from the abdominal cavity.

The thoracic cavity is divided into 2 **lateral compartments:** the lungs and their covering of serous membranes, and a **central compartment** called the **mediastinum** which contains most of the viscera of the thorax.

Clinical Correlate

Passage of instruments through the intercostal space is done in the **lower part** of the space to avoid the intercostal neurovascular structures (as during a thoracentesis).

An intercostal nerve block is done in the **upper portion** of the intercostal space.

Intercostal Spaces

- There are 11 **intercostal spaces** within the thoracic wall (Figure II-2-4A). The spaces are filled in by 3 layers of intercostal muscles and their related fasciae and are bounded superiorly and inferiorly by the adjacent ribs.
- The **costal groove** is located along the inferior border of each rib (upper aspect of the intercostal space) and provides protection for the intercostal nerve, artery, and vein which are located in the groove. The vein is most superior and the nerve is inferior in the groove (VAN).
- The intercostal arteries are contributed to anteriorly from branches of the internal thoracic artery (branch of the subclavian artery) and posteriorly from branches of the thoracic aorta. Thus, the intercostal arteries can provide a potential collateral circulation between the subclavian artery and the thoracic aorta.

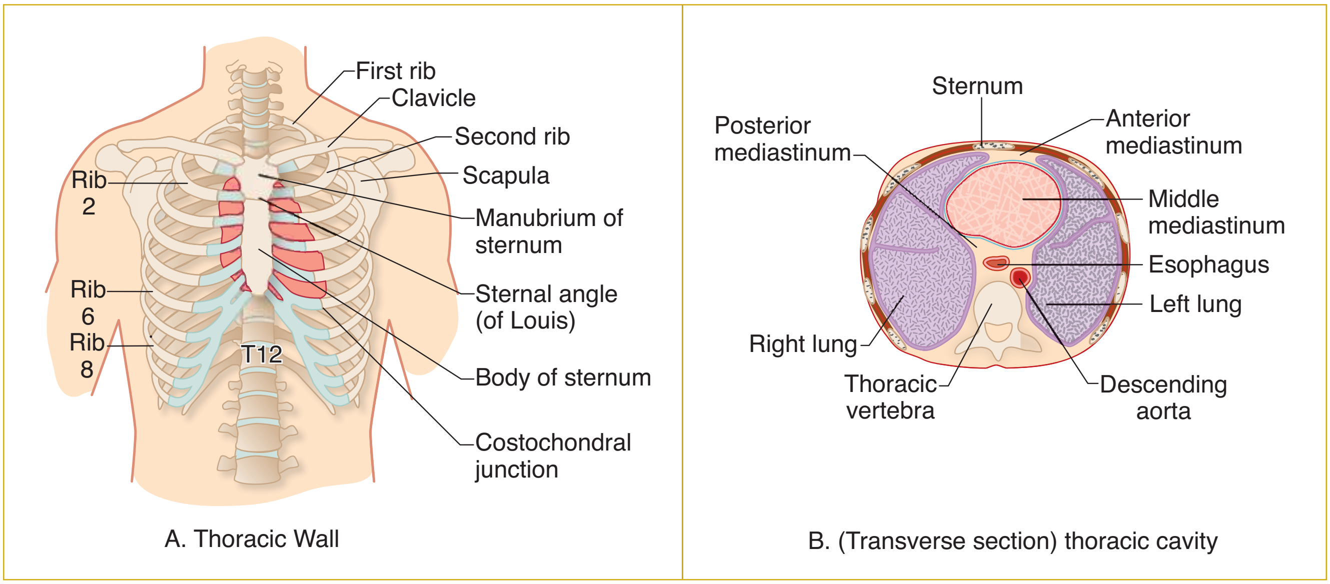

Figure II-2-4. Thoracic Cavity

PLEURA AND PLEURAL CAVITY

Within the thoracic and abdominal cavities there are 3 serous mesodermal-derived membranes which form a covering for the lungs (**pleura**), heart (**pericardium**), and abdominal viscera (**peritoneum**).

Each of these double-layered membranes permits friction-reducing movements of the viscera against adjacent structures.

The outer layer of the serous membranes is referred to as the **parietal layer;** and the inner layer which is applied directly to the surface of the organ is called the **visceral layer**. The 2 layers are continuous and there is a potential space (pleural cavity) between the parietal and visceral layers containing a thin layer of serous fluid.

Pleura

The **pleura** is the serous membrane that invests the lungs in the lateral compartments of the thoracic cavity (Figure II-2-5). The external **parietal pleura** lines and attaches to the inner surfaces of the chest wall, diaphragm, and mediastinum. The innermost **visceral** layer reflects from the parietal layer at the hilum of the lungs and is firmly attached to and follows the contours of the lung. Visceral and parietal pleura are continuous at the root of the lung.

The **parietal pleura** is regionally named by its relationship to the thoracic wall and mediastinum (Figure II-2-5):

- **Costal parietal pleura** is lateral and lines the inner surfaces of the ribs and intercostal spaces
- **Diaphragmatic parietal pleura** lines the thoracic surface of the diaphragm

Clinical Correlate

Respiratory distress syndrome is caused by a deficiency of surfactant (type II pneumocytes). This condition is associated with premature infants, infants of diabetic mothers, and prolonged intrauterine asphyxia. Thyroxine and cortisol treatment increase the production of surfactant.

Surfactant deficiency may lead to **hyaline membrane disease,** whereby repeated gasping inhalations damage the alveolar lining. Hyaline membrane disease is characterized histologically by collapsed alveoli (atelectasis) and eosinophilic (pink) fluid covering the alveoli.

- **Mediastinal parietal pleura** is medial and lines the mediastinum. The mediastinal pleura reflects and becomes continuous with the visceral pleura at the hilum.
- **Cervical parietal pleura** extends into the neck above the first rib where it covers the apex of the lung.

The **visceral pleura** tightly invests the surface of the lungs, following all of the fissures and lobes of the lung.

Innervation of Pleura

The **parietal pleura** has extensive **somatic** sensory innervation provided by nerves closely related to different aspects of the pleura.

- The **intercostal nerves** supply the **costal** and peripheral portions of the **diaphragmatic** pleura.
- The **phrenic nerve** supplies the central portion of the **diaphragmatic** pleura and the **mediastinal** pleura.

The **visceral pleura** is supplied by visceral sensory nerves that course with the autonomic nerves.

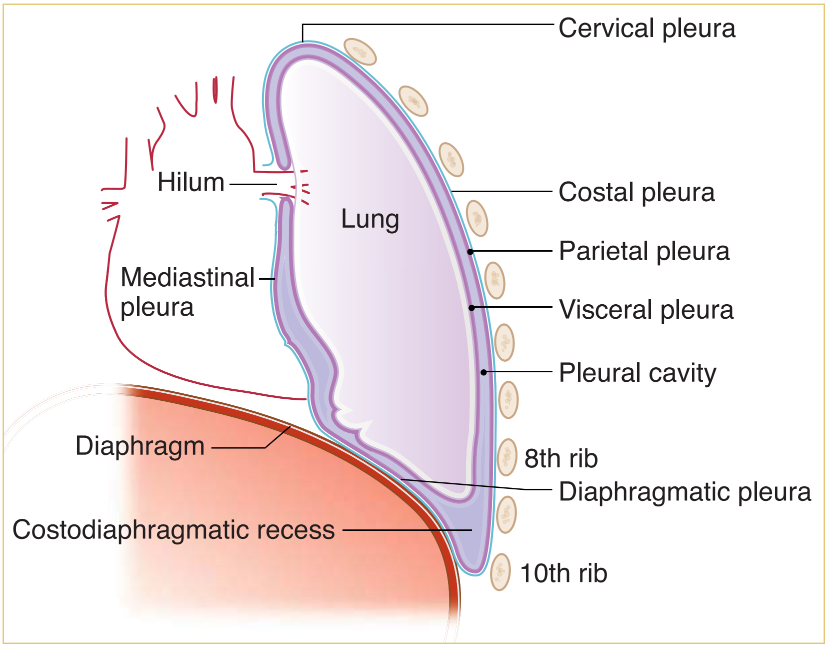

Figure II-2-5. Layers of the Pleura

The **pleural cavity** is the potential space between the parietal and visceral layers of the pleura. It is a closed space which contains a small amount of serous fluid that lubricates the opposing parietal and visceral layers.

The introduction of air into the pleural cavity may cause the lung to collapse, resulting in a **pneumothorax** which causes shortness of breath and painful respiration. The lung collapses due to the loss of the negative pressure of the pleural cavity during a pneumothorax.

Clinical Correlate

Inflammation of the parietal pleural layers (**pleurisy**) produces sharp pain upon respiration. Costal inflammation produces local dermatome pain of the chest wall via the intercostal nerves; whereby mediastinal irritation produces referred pain via the phrenic nerve to the shoulder dermatomes of C3–5.

Clinical Correlate

Open pneumothorax occurs when air enters the pleural cavity following a penetrating wound of the chest cavity. Air moves freely through the wound during inspiration and expiration. During inspiration, air enters the chest wall and the mediastinum will shift toward other side and compress the opposite lung. During expiration, air exits the wound and the mediastinum moves back toward the affected side.

Tension pneumothorax occurs when a piece of tissue covers and forms a flap over the wound. During inspiration, air enters the chest cavity, which results in a shift of the mediastinum toward the other side, compressing the opposite lung. During expiration, the piece of tissue prevents the air from escaping the wound, which increases the pressure and the shift toward the opposite side is enhanced. This severely reduces the opposite lung function and venous return to the heart and can be life-threatening.

Pleural Reflections

Pleural reflections are the areas where the parietal pleura abruptly changes direction from one wall to the other, outlining the extent of the pleural cavities.

- The sternal line of reflection is where the costal pleura is continuous with the mediastinal pleura posterior to the sternum (from costal cartilages 2–4). The pleural margin then passes inferiorly to the level of the sixth costal cartilage.
- Around the chest wall, there are **2 rib interspaces** separating the inferior limit of parietal pleural reflections from the inferior border of the lungs and visceral pleura: between ribs 6–8 in the **midclavicular line**, ribs 8–10 in the **midaxillary line**, and ribs 10–12 at the vertebral column (**paravertebral line**), respectively.

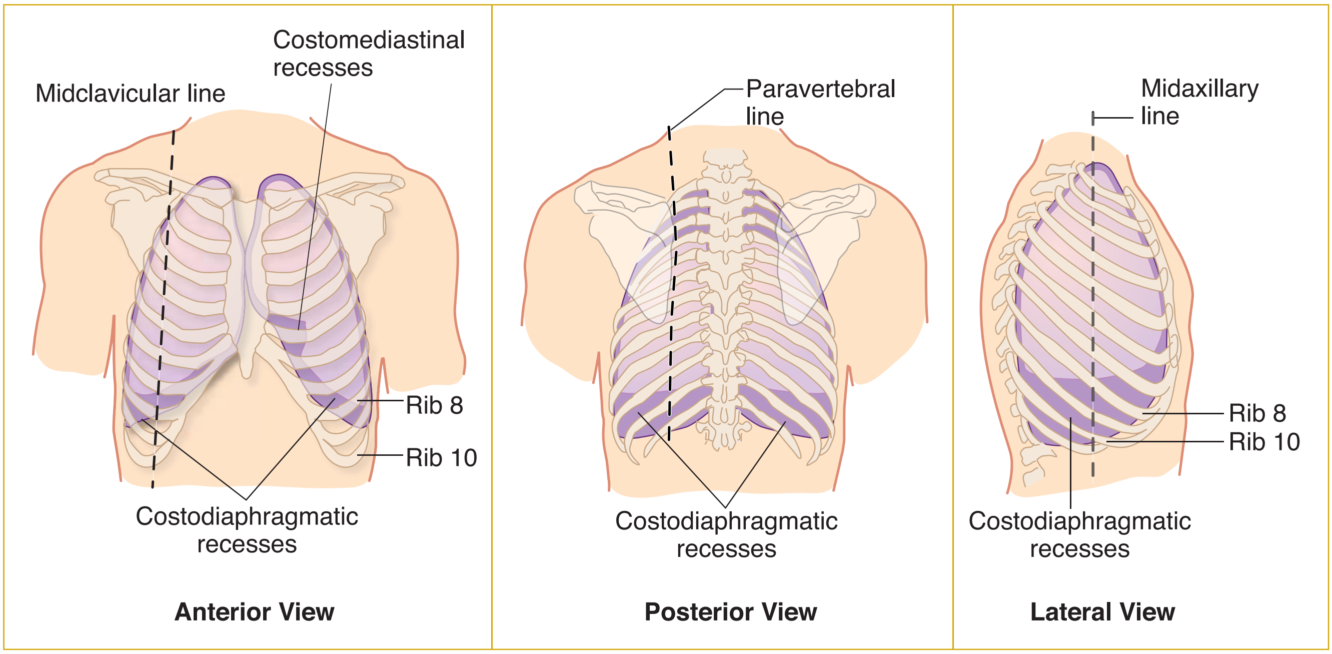

Figure II-2-6. Pleural Reflections and Recesses

Pleural Recesses

Pleural recesses are potential spaces not occupied by lung tissue except during deep inspiration.

- **Costodiaphragmatic recesses** are spaces below the inferior borders of the lungs where costal and diaphragmatic pleura are in contact.
- The **costomediastinal recess** is a space where the left costal and mediastinal parietal pleura meet, leaving a space caused by the cardiac notch of the left lung. This space is occupied by the lingula of the left lung during inspiration.

Note

	Visceral Pleura	Parietal Pleura
Midclavicular line	6th rib	8th rib
Midaxillary line	8th rib	10th rib
Paravertebral line	10th rib	12th rib

LUNGS

The lungs and the pleural membranes are located in the lateral compartment of the thoracic cavity. The lungs are separated from each other in the midline by the mediastinum. The **hilum** of the lung is on the medial surface and serves for passage of structures in the root of the lung: the pulmonary vessels, primary bronchi, nerves, and lymphatics.

Surfaces and Regions

Each lung has 3 surfaces:

- The **costal surface** is smooth and convex and is related laterally to the ribs and tissues of the chest wall.
- The **mediastinal surface** is concave and is related medially to the middle mediastinum and the heart. The mediastinal surfaces contain the **root** of the lung and a deep **cardiac impression**, more pronounced on the left lung.
- The **diaphragmatic surface** (base) is concave and rests on the superior surface of the diaphragm. It is more superior on the right owing to the presence of the liver.

Clinical Correlate

A tumor at the apex of the lung (Pancoast tumor) may result in thoracic outlet syndrome.

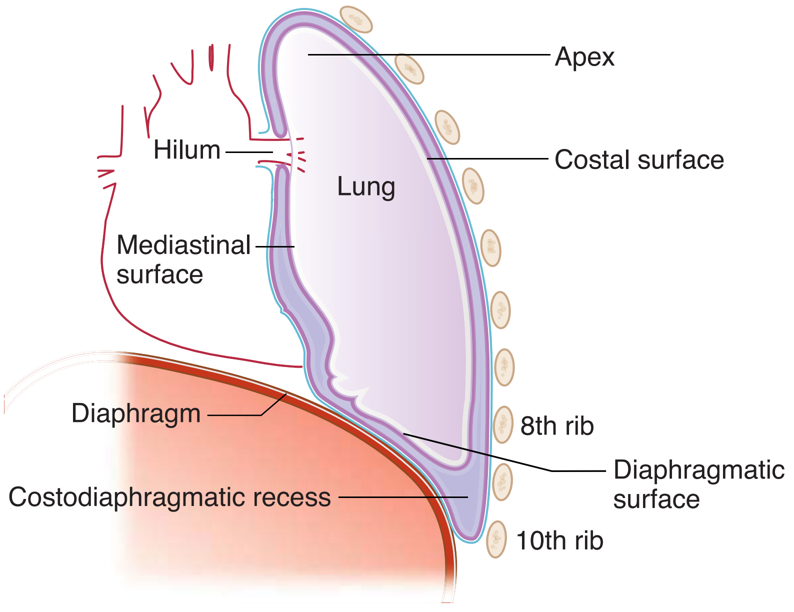

Figure II-2-7. Surfaces of the Lung

The **apex** (cupola) of the lung projects superiorly into the root of the neck above the level of the first rib and is crossed anteriorly by the subclavian artery and vein.

Lobes and Fissures

The **right lung** is divided into 3 lobes (**superior, middle, inferior**) separated by 2 fissures, the **horizontal** and **oblique fissures**. The horizontal fissure separates the superior from the middle lobe and the oblique fissure separates the middle from the inferior lobe.

The **left lung** is divided into 2 lobes (**superior, inferior)** separated by an **oblique fissure**. The **lingula** of the upper lobe of the left lung corresponds to the middle lobe of the right lung.

- The **oblique fissure** of both lungs projects anteriorly at approximately the **5th intercostal space** in the midclavicular line, ending medially deep to the 6th costal cartilage.
- The **horizontal fissure** runs horizontally from the oblique fissure in the **right 5th intercostal space** to the **right 4th costal cartilage**.

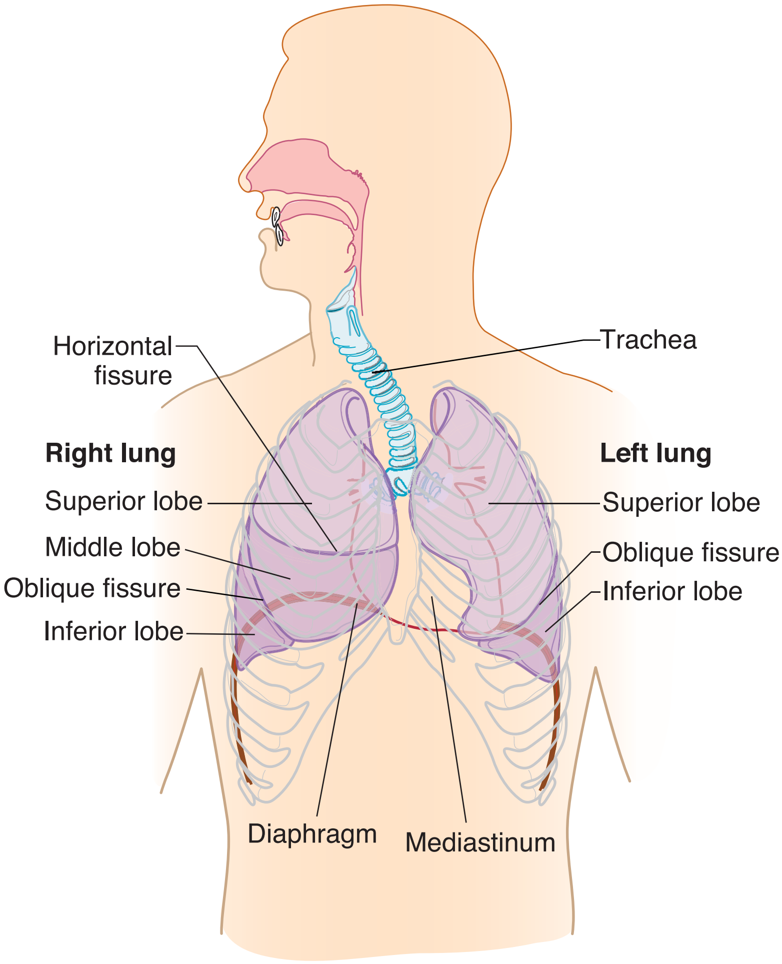

Figure II-2-8. Lobes and Fissures of Lungs

Lymphatic System

The **lymphatic system** consists of an extensive network of lymph capillaries, vessels, and nodes that drain extracellular fluid from most of the body tissues and organs. The lymph flow will return to the blood venous system by 2 major lymphatic vessels, the **right lymphatic duct** and the **thoracic duct** on the left (Figure II-2-10A). These 2 vessels drain into the junction of the internal jugular and the subclavian veins on their respective sides.

Clinical Correlate

The superior lobe of the **right lung** projects anteriorly on the chest wall **above** the **4th rib** and the middle lobe projects anteriorly **below** the 4th rib.

A small portion of the inferior lobe of both lungs projects **below** the 6th rib anteriorly but primarily projects to the **posterior chest** wall.

Clinical Correlate

- To listen to **breath sounds** of the **superior lobes** of the right and left lungs, the stethoscope is placed on the superior area of the anterior chest wall (**above the 4th rib** for the right lung).
- For **breath sounds** from the **middle lobe of the right lung**, the stethoscope is placed on the anterior chest wall **inferior to the 4th rib** and medially toward the sternum.
- For **the inferior lobes** of both lungs**, breath sounds** are primarily heard on the **posterior** chest wall.

Clinical Correlate

Aspiration of a foreign body will more often enter the **right primary bronchus**, which is shorter, wider, and more vertical than the left primary bronchus. When the individual is vertical, the foreign body usually falls into the **posterior basal segment** of the **right inferior lobe**.

- The **thoracic duct** carries all lymphatic drainage from the body below the diaphragm and on the left side of the trunk and head above the diaphragm (Figure II-2-10B).
- **The right lymphatic duct** drains lymph flow from the right head and neck and the right side of the trunk above the diaphragm (Figure II-2-10B).

Lymphatic Drainage

The lymphatic drainage of the lungs is extensive and drains by way of **superficial** and **deep** lymphatic plexuses. The superficial plexus is immediately deep to the visceral pleura. The deep plexus begins deeply in the lungs and drains through **pulmonary nodes** which follow the bronchial tree toward the hilum.

The major nodes involved in the lymphatic drainage of these 2 plexuses are:

- **Bronchopulmonary (hilar) nodes** are located at the hilum of the lungs. They receive lymph drainage from both superficial and deep lymphatic plexuses, and they drain into the tracheobronchial nodes.
- **Tracheobronchial nodes** are located at the bifurcation of the trachea, and they drain into the right and left bronchomediastinal nodes and trunk.
- **Bronchomediastinal nodes** and trunk are located on the right and left sides of the trachea, and they drain superiorly into either the right lymphatic duct or the thoracic duct on the left.

Clinical Correlate

The lymphatic drainage from the **lower lobe of the left lung** also drains across the midline into the **right bronchomediastinal** lymphatic trunk and nodes, then continues along the right pathway to the right lymphatic duct. This is important to consider with metastasis of lung cancer.

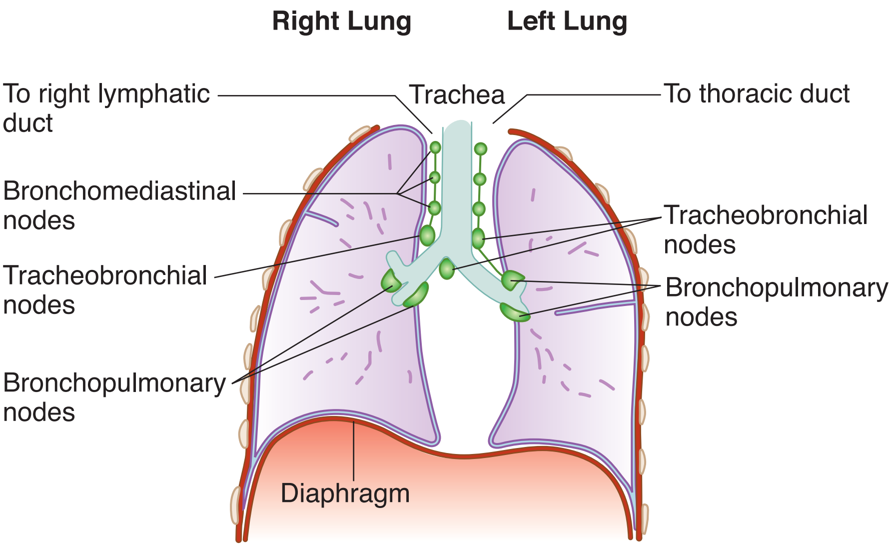

Figure II-2-9. Lymphatics of the Lungs

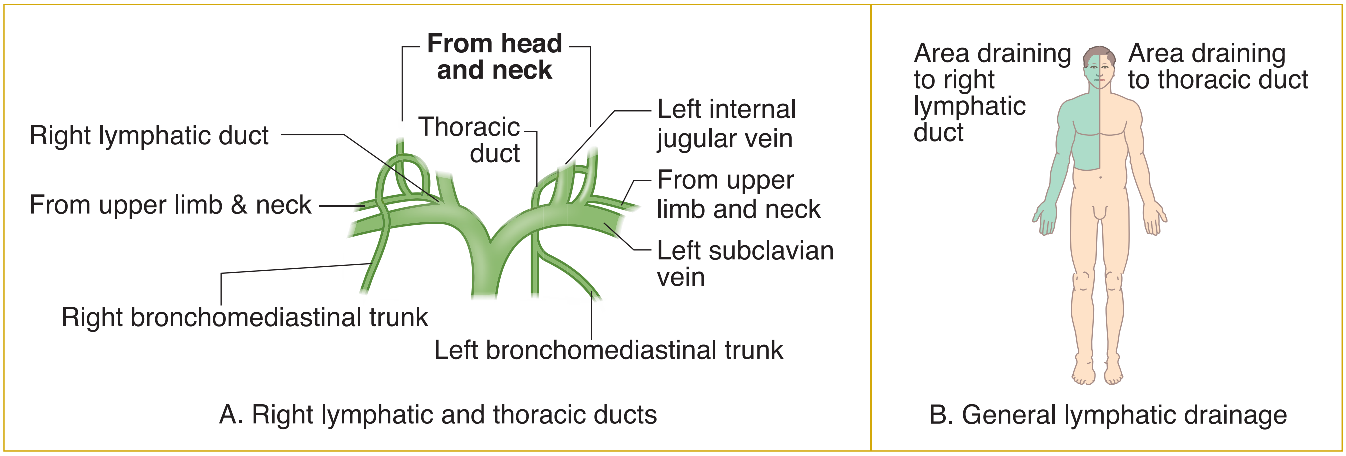

Figure II-2-10. Lymphatic Drainage

RESPIRATORY HISTOLOGY

The lung is an organ that functions in the intake of oxygen and exhaling of CO_2. Approximately 14 times each minute, we take in about 500 mL of air per breath. Inspired air will be spread over 120 square meters of the surface area of the lungs. The air–blood barrier has to be thin enough for air to pass across but tough enough to keep the blood cells inside their capillaries.

Because lungs are opened to the outside world, they are susceptible to environmental insults in the form of pollution and infectious bacteria.

The lungs receive the entire cardiac output and are positioned to modify various blood components. The pulmonary endothelium plays an active role in the metabolic transformation of lipoproteins and prostaglandins. **The enzyme that converts angiotensin I to angiotensin II is produced by the lung endothelial cells.**

Clinical Correlate

Any disease that affects capillaries also affects the extensive capillary bed of the lungs. Bacteria which colonize the lungs may damage the barriers between the alveoli and the capillaries, gaining access to the bloodstream (a common complication of bacterial pneumonia).

- With allergies, smooth-muscle constriction reduces the diameter of air tubes and results in reduced air intake.
- Lung cancers commonly develop from bronchi (smoking, asbestos, and excessive radiation are the main causes).
- Mesothelioma is a malignant tumor of the pleura (causative agent: asbestos dust).

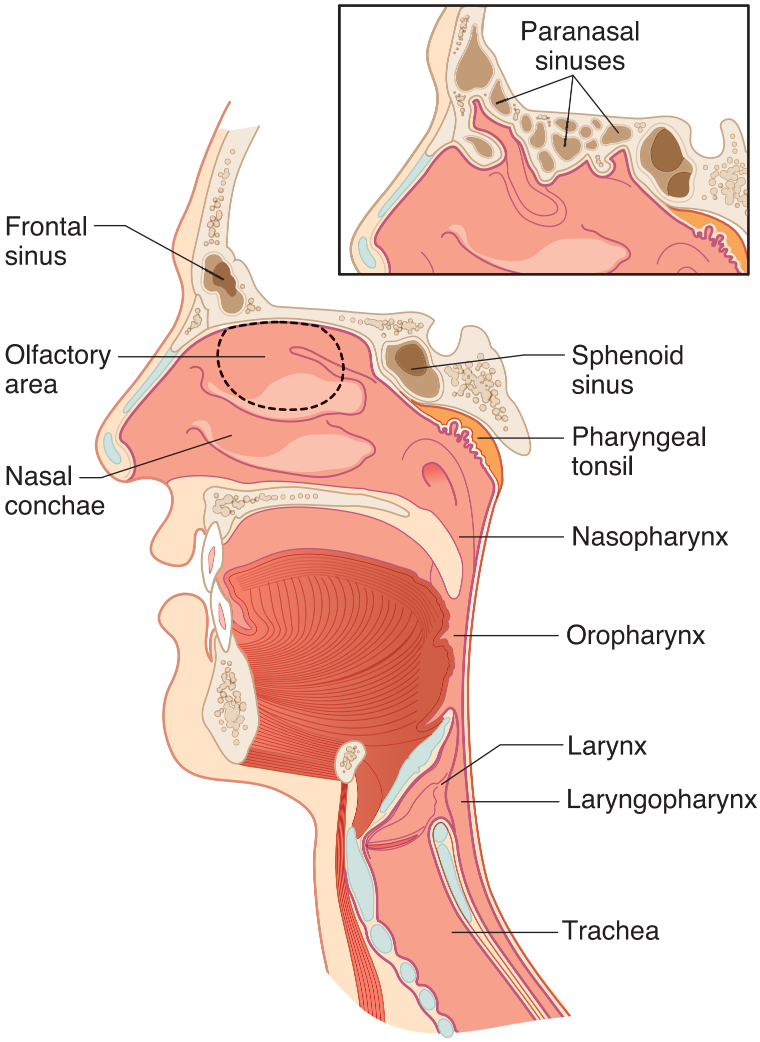

Figure II-2-11. Respiratory Pathways

Table II-2-1. Histologic Features of Trachea, Bronchi, and Bronchioles

	Trachea	Bronchi	Bronchioles
Epithelia	Pseudostratified ciliated columnar (PCC) cells, goblet cells	PCC to simple columnar cells	Ciliated, some goblet cells, Clara cells in terminal bronchioles
Cartilage	16–20 C-shaped cartilaginous rings	Irregular plates	None
Glands	Seromucous glands	Fewer seromucous glands	None
Smooth muscle	Between open ends of C-shaped cartilage	Prominent	Highest proportion of smooth muscle in the bronchial tree
Elastic fibers	Present	Abundant	Abundant

TRACHEA

The **trachea** is a hollow tube, about 10 cm in length (and about 2 cm in diameter), extending from the larynx to its bifurcation at the carina to form a primary bronchus for each lung. The most striking structures of the trachea are the C-shaped hyaline cartilage rings. In the human there are 16–20 of them distributed along the length of the trachea. The rings overlap in the anterior part of the trachea. The free posterior ends of the C-shaped cartilages are interconnected by smooth-muscle cells.

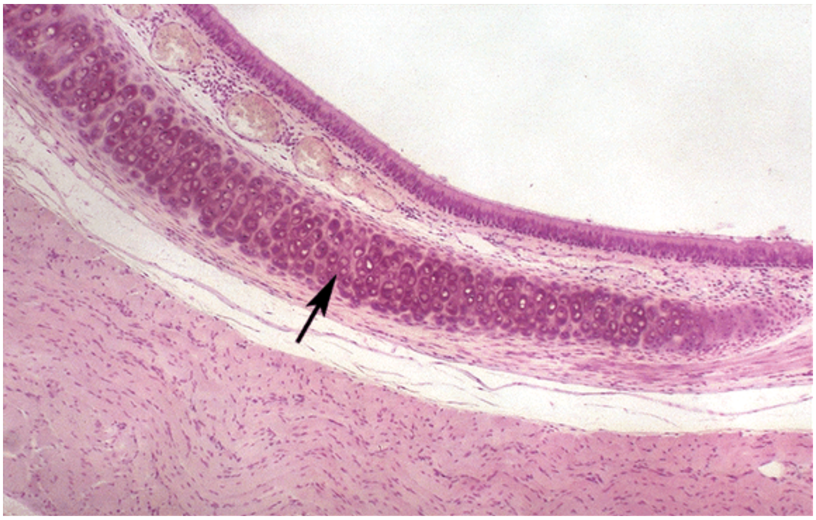

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Figure II-2-12. Trachea with a hyaline cartilage ring (arrow) and pseudostratified columnar epithelium

The trachea is composed of concentric rings of mucosa, submucosa, an incomplete muscularis, and an complete adventitia.

- The mucosa has 3 components: a pseudostratified epithelium, an underlying vascularized loose connective tissue (lamina propria) that contains immune cells, and a thin layer of smooth-muscle cells (muscularis mucosa).
- The submucosa is a vascular service area containing large blood vessels. Collagen fibers, lymphatic vessels and nerves are also present in this layer.
- The outside covering of the trachea, the adventitia, is composed of several layers of loose connective tissue.

The epithelial lining of the trachea and bronchi is pseudostratified columnar in which all cells lie on the same basal membrane but only some reach the luminal surface. The only other place in the body with this epithelium is the male reproductive tract.

Clinical Correlate

If mucosal clearance is ineffective, or the mechanism overwhelmed, infection (pathogenic bacteria) or pneumoconiosis (dust-related disease) may follow.

In cystic fibrosis, the secreted mucous is thick or viscous and the cilia have a difficult time moving it toward the pharynx. Patients with this disease have frequent infections of the respiratory system.

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Figure II-2-13. Pseudostratified Columnar Epithelium with Goblet Cells (arrowhead) Surrounded by Ciliated Cells (arrow)

Clinical Correlate

Patients lacking dynein have immotile cilia or **Kartagener syndrome**.

With immotile cilia, patients are subject to many respiratory problems because their cilia cannot move this mucous layer with its trapped bacteria. Males also possess immotile sperm.

Tracheal Epithelial Cell Types

Columnar cells extend from the basal membrane to the luminal surface. These cells contain 200–300 apical cilia per cell that are intermingled with microvilli. The cilia are motile and beat to help move the secreted mucous layer over the lining of the trachea and out of the respiratory system.

Goblet cells secrete a polysaccharide mucous material into the lumen of trachea. Mucous production is supplemented by secretions of the submucosal mixed glands. The mucous layer of the respiratory system traps particulate substances (dust, bacteria, and viruses) and absorbs noxious water-soluble gases such as ozone and sulfur dioxide. The mucous sticky layer is moved by the beating cilia toward the pharynx where it is swallowed. This movement is known as the mucociliary escalator system. Most material (dust and bacteria) is trapped in the mucous layer, and is removed and digested.

Pulmonary neuroendocrine (PNE) cells are comparable to the endocrine cells in the gut. These epithelial neuroendocrine cells have been given various names:

- **APUD cells** (**A**mino-**P**recursor-**U**ptake-**D**ecarboxylase), **DNES cells** (**D**iffuse **N**euro **E**ndocrine **S**ystem) and **K** (Kulchitsky) **cells.** These cells occur in clusters and are often located at airway branch points.
- **Brush cells** may represent goblet cells that have secreted their products or intermediate stages in the formation of goblet or the tall ciliated cells. They have short microvilli on their apical surfaces. Some of these cells have synapses with intraepithelial nerves, suggesting that these cells may be sensory receptors.

Basal cells are stem cells for the ciliated and goblet cells. The stem cells lie on the basal membrane but do not extend to the lumen of the trachea. These cells, along with the epithelial neuroendocrine cells, are responsible for the pseudostratified appearance of the trachea.

BRONCHI

The **bronchial tree** forms a branching airway from the trachea to the bronchioles. When the primary bronchi enter the lung, they give rise to 5 secondary or lobar bronchi—3 for the right lung and 2 for the left. The 5 lobes are further subdivided into 10 tertiary or segmental bronchi in each lung, which form bronchopulmonary segments.

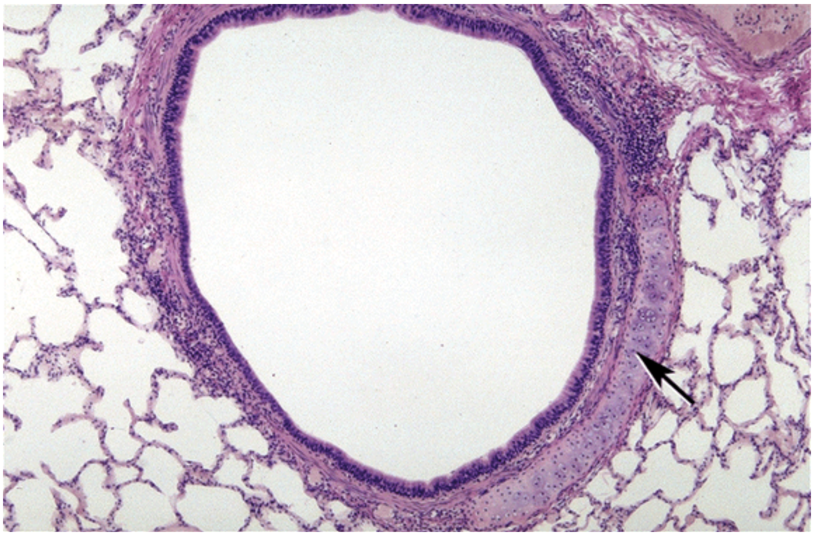

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Figure II-2-14. Bronchus with a Plate of Cartilage (arrow)

The epithelial lining of the bronchi is also pseudostratified. It consists of **ciliated columnar cells, basal cells, mucous cells, brush cells and neuroendocrine** (APUD, DNES, or K) **cells**. There are also seromucous glands in the submucosa that empty onto the epithelial surface via ducts. The walls of bronchi contain irregular plates of cartilage and circular smooth-muscle fascicles bound together by elastic fibers. The number of goblet cells and submucosa glands decreases from the trachea to the small bronchi.

BRONCHIOLES

The **wall of a bronchiole** does not contain cartilage or glands. The smooth-muscle fascicles are bound together by elastic fibers. The epithelium is still ciliated, but is a simple cuboidal or columnar epithelium rather than pseudostratified. The epithelial lining of the airway is composed of **ciliated cells** (goblet and basal cells are absent in the terminal bronchioles) and an additional type called the **Clara cell.**

Clinical Correlate

The columnar and goblet cells are sensitive to irritation. The ciliated cells become taller, and there is an increase in the number of goblet cells and submucosal glands.

Intensive irritation from smoking leads to a squamous metaplasia where the ciliated epithelium becomes a squamous epithelium. This process is reversible.

Clinical Correlate

Bronchial metastatic tumors arise from Kulchitsky cells.

Clinical Correlate

Cystic fibrosis can result in abnormally thick mucous, in part due to defective chloride transport by Clara cells.

Clara cells (also called bronchiolar secretory cells) are nonciliated and **secrete a serous solution similar to surfactant**. They aid in the **detoxification of airborne toxins**, and serve as a **stem cell for the ciliated cells** and for themselves. The number of Clara cells increases in response to increased levels of pollutants like cigarette smoke. **Clara cells are most abundant in the terminal bronchioles, where they make up about 80% of the epithelial cell lining;** they are also involved with chloride ion transport into the lumens of the terminal bronchioles.

Clinical Correlate

Chronic obstructive pulmonary disease (COPD) affects the bronchioles and includes emphysema and asthma.

- Emphysema is caused by a loss of elastic fibers and results in chronic airflow obstruction.
- Asthma is a chronic process characterized by a reversible narrowing of airways.
- Asthma is reversible; emphysema is not.

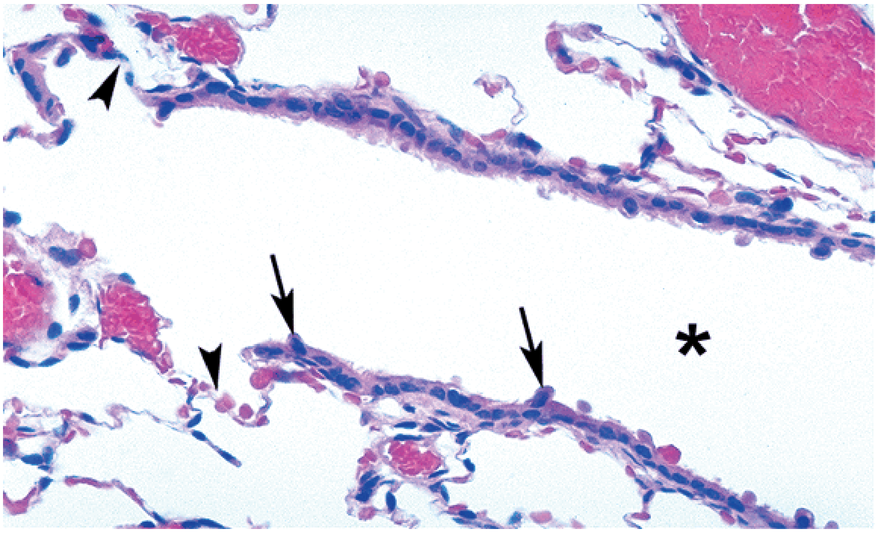

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Figure II-2-15. Terminal bronchiole lumen (asterisk) with epithelium containing ciliated cells and Clara cells (arrows)

The **terminal bronchiole** is the last conducting bronchiole. This bronchiole is followed by **respiratory bronchioles** which are periodically interrupted by alveoli in their walls. The goblet cells are absent from the epithelial lining of the respiratory bronchioles; however, this epithelium is still lined with a sparse ciliated cuboidal epithelium which prevents the movement of mucous into the alveoli. After the last respiratory bronchiole, the wall of the airway disappears and air enters the alveoli.

ALVEOLAR DUCTS, ALVEOLAR SACS, AND THE ALVEOLI

The **alveolar ducts and sacs** have little or no walls and consist almost entirely of alveoli. The alveoli constitute 80–85% of the volume of the normal lung. There are 300 million alveoli in the lungs, each ~200 microns in diameter. The cuboidal epithelium of the respiratory bronchioles and the alveolar ducts are continuous with the squamous cells lining the alveoli.

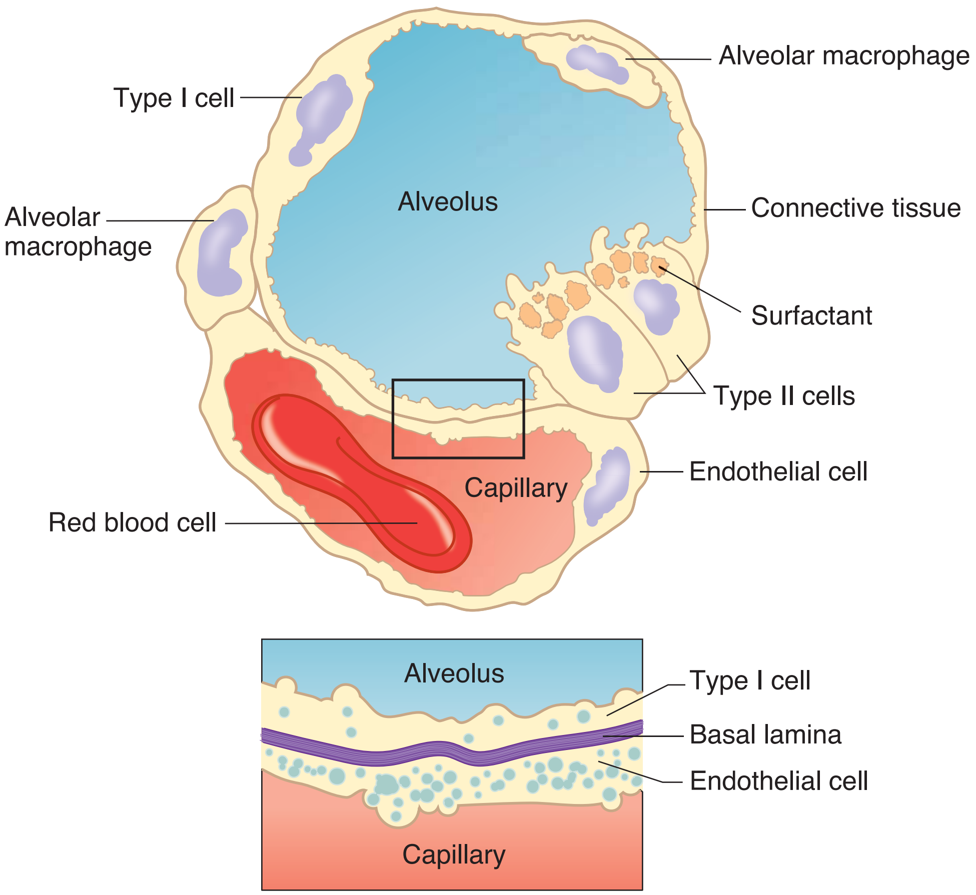

Figure II-2-16. Alveolus and Blood–Air Barrier

The **type I pneumocyte** is the major cell lining cell of the alveolar surfaces (also called small alveolar cell or alveolar type I cell).

- Represent only 40% of the alveolar lining cells, but are spread so thinly they cover 90–95% of the surface
- Primarily involved in gas exchange
- Post-mitotic

The **type II pneumocyte** is the other major alveolar cell (also called great alveolar cell [because of its size], granular pneumocyte, septal cell, corner cell, niche cell, or alveolar type II).

- Constitute 60% of the cell lining the alveoli, but form only 5–10% of the surface
- Produce and secrete surfactant
- Large, round cells with "myelin figures" in their apical cytoplasm which represent the remnants of surfactant after histological processing
- Serve as stem cells for themselves and the type I cell

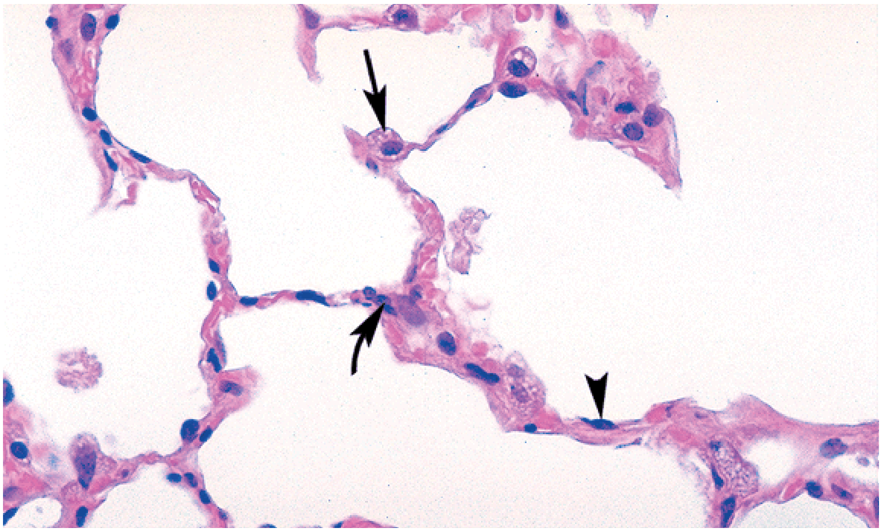

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Figure II-2-17. Alveoli with Type I Pneumocytes (arrowhead), Type II Pneumocytes (arrow), and Alveolar Macrophage (curved arrow) in the Alveolar Wall

Surfactant

Surfactant is essential to maintain the normal respiratory mechanics of the alveoli. Production of surfactant in the fetus is essential for the survival of the neonate as it takes its first breath. Surfactant is composed of a mixture of phospholipids and surfactant proteins whose function is to aid in the spreading of the surfactant at the alveolar air–water interface. The phospholipids act as a detergent which lowers the surface tension of the alveoli and prevents alveolar collapse during expiration.

Most surfactant is recycled back to Type II cells for reutilization; some of it undergoes phagocytosis by macrophages.

Clinical Correlate

Corticosteroids induce the fetal synthesis of surfactant. High insulin levels in diabetic mothers antagonize the effects of corticosteroids.

Infants of diabetic mothers have a higher incidence of respiratory distress syndrome.

Alveolar Wall

In the **alveolar wall** under the alveolar epithelium is a rich network of capillaries arising from pulmonary arteries. The alveolar wall contains a variety of cells and extracellular fibers. The cells include fibroblasts, macrophages, myofibroblasts, smooth-muscle cells, and occasional mast cells. Type I and II collagens, as well as elastic fibers, are in the septa. Type I collagen is present primarily in the walls of the bronchi and bronchioles. Twenty percent of the mass of the lung consists of collagen and elastic fibers. Elastic fibers are responsible for the stretching and recoiling activities of the alveoli during respiration. These microscopic elements are responsible for the recoil of the lungs during expiration.

Gas exchange occurs between capillary blood and alveolar air across the blood–gas barrier. This barrier consists of surfactant, the squamous Type I pneumocytes, a shared basal lamina, and capillary endothelium. The distance between the lumen of the capillary and the lumen of the alveolus can be as thin as 0.1 microns. There are openings in the wall of most alveoli that form the **pores of Kohn**. These pores are thought to be important in collateral ventilation. The diameter of these alveolar pores can be as large as 10 to 15 microns.

Alveolar Macrophages

The **alveolar macrophages** are derived from monocytes that exit the blood vessels in the lungs. The resident alveolar macrophages can undergo limited mitoses to form additional macrophages. These cells can reside in the interalveolar septa as well as in the alveoli. Alveolar macrophages that patrol the alveolar surfaces may pass through the pores of Kohn.

There are ~1–3 macrophages per alveolus. Alveolar macrophages vary in size, 15–40 microns in diameter. These macrophages represent the last defense mechanism of the lung. Macrophages can pass out of the alveoli to the bronchioles and enter the lymphatics or become trapped in the moving mucous layer and propelled toward the pharynx to be swallowed and digested.

Clinical Correlate

Alveolar macrophages have several other names: **dust cells** because they have phagocytosed dust or cigarette particles, and **heart failure cells** because they have phagocytosed blood cells that have escaped into the alveolar space during congestive heart failure.

EMBRYOLOGY OF THE HEART

Formation of Heart Tube

The heart begins to develop from splanchnic **mesoderm** in the latter half of week 3 within the cardiogenic area of the cranial end of the embryo. **Neural crest cells** migrate into the developing heart and play an important role in cardiac development. The cardiogenic cells condense to form a pair of primordial heart tubes which will fuse into a single **heart tube** during body folding.

- The heart tube undergoes dextral looping (bends to the right) and rotation.
- The upper truncus arteriosus (ventricular) end of the tube grows more rapidly and folds downward and **ventrally** and to the right.
- The atria and sinus venosus lower part of the tube fold upward and **dorsally** and to the left. These foldings begin to place the chambers of the heart in their postnatal anatomic positions.

The **primitive heart tube forms 4 dilatations and a cranial outflow tract**, the truncus arteriosus. The fates of these are shown below.

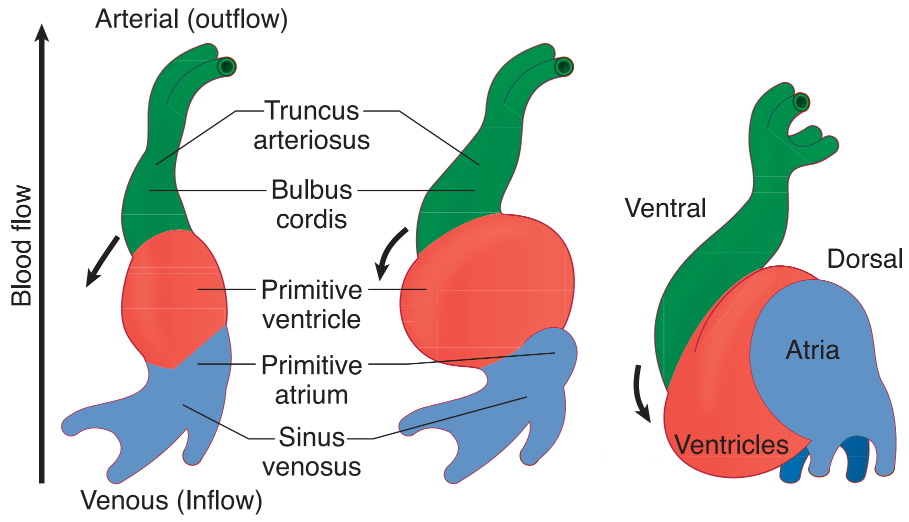

Figure II-2-18. Development of the Heart Tube

Table II-2-2. Adult Structures Derived From the Dilatations of the Primitive Heart

Embryonic Dilatation	Adult Structure
Truncus arteriosus (neural crest)	Aorta; Pulmonary trunk; Semilunar values
Bulbus cordis	Smooth part of right ventricle (**conus arteriosus**) Smooth part of left ventricle (**aortic vestibule**)
Primitive ventricle	Trabeculated part of right ventricle Trabeculated part of left ventricle
Primitive atrium*	Trabeculated part of right atrium (pectinate muscles) Trabeculated part of left atrium (pectinate muscles)
Sinus venosus (the only dilation that does not become subdivided by a septum)	Right—Smooth part of right atrium (**sinus venarum**) Left—Coronary sinus and oblique vein of left atrium

*The **smooth-walled** part of the **left atrium** is formed by incorporation of parts of the **pulmonary veins** into its wall. The **smooth-walled part** of the **right atrium** is formed by the incorporation of the **right sinus venosus.**

Fetal Circulation

There are **3 major venous systems** that flow into the sinus venosus end of the heart tube:

- **Vitelline (omphalomesenteric) veins** drain deoxygenated blood from the yolk stalk; they will coalesce and form the veins of the liver (sinusoids, hepatic portal vein, hepatic vein) and part of the inferior vena cava.
- **Umbilical vein** carries oxygenated blood from the placenta.
- **Cardinal veins** carry deoxygenated blood from the body of the embryo; they will coalesce and contribute to some of the major veins of the body (brachiocephalic, superior vena cava, inferior vena cava, azygos, renal).

During fetal circulation, **oxygenated blood flood** from the placenta to the fetus passes through the **umbilical vein**. Three **vascular shunts** develop in the fetal circulation to bypass blood flow around the liver and lungs:

- The **ductus venosus** allows oxygenated blood in the umbilical vein to bypass the sinusoids of the liver into the inferior vena cava and to the right atrium. From the right atrium, oxygenated blood flows mostly through the foramen ovale into the left atrium then left ventricle and into the systemic circulation.
- The **foramen ovale** develops during atrial septation to allow oxygenated blood to bypass the pulmonary circulation. Note that this is a **right-to-left** shunting of blood during fetal life.

- During fetal circulation, the superior vena cava drains deoxygenated blood from the upper limbs and head into the right atrium. Most of this blood flow is directed into the right ventricle and into the pulmonary trunk. The **ductus arteriosus** opens into the underside of the aorta just **distal** to the origin of the left subclavian artery and shunts this deoxygenated blood from the pulmonary trunk to the aorta to bypass the pulmonary circulation.

The shunting of blood through the foramen ovale and through the ductus arteriosus (right to left) during fetal life occurs because of a **right-to-left pressure gradient**.

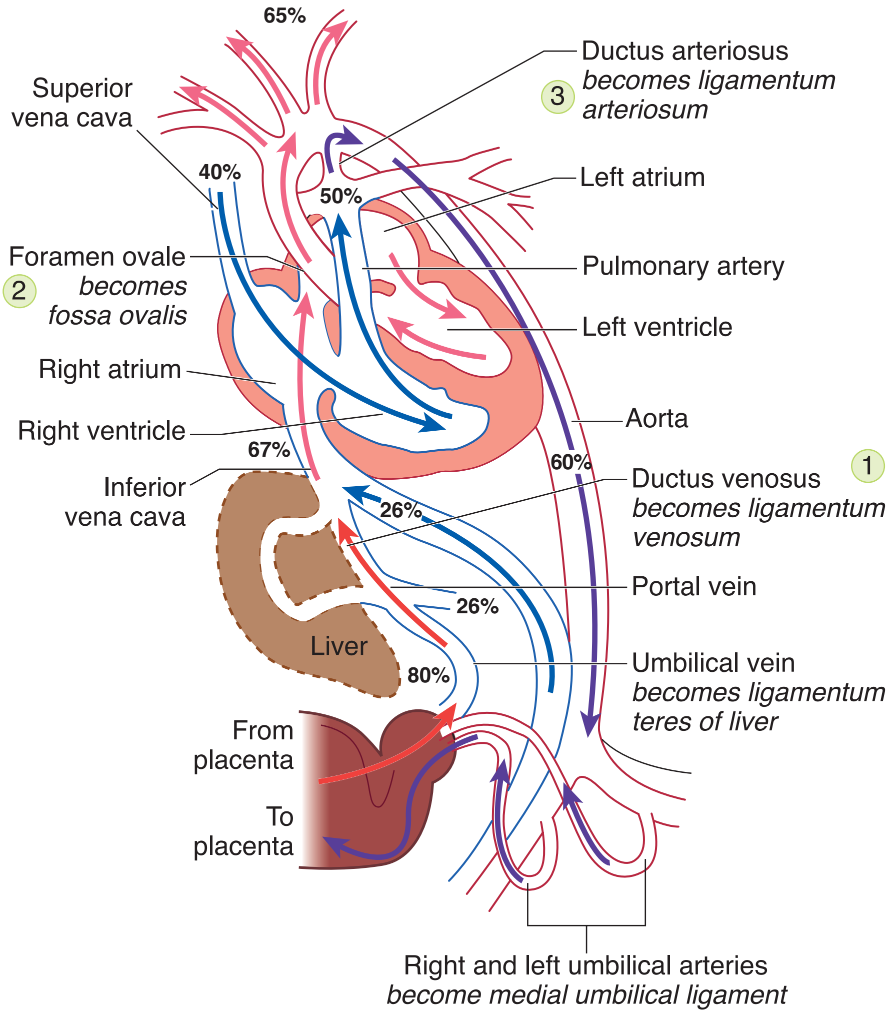

Figure II-2-19. Fetal Circulation and Shunts

Following birth, these 3 shunts, labelled **1**, **2**, and **3**, will close because of changes in the pressure gradients and in oxygen tensions. The umbilical vein closes and reduces blood flow into the right atrium. The ductus venosus also closes. Lung expansion reduces pulmonary resistance and results in increased flow to the lungs and increased venous return to the left atrium.

Pressure Gradients

Fetal

R → L

Postnatal

L → R

- Closure of the foramen ovale occurs as a result of the increase in left atrial pressure and reduction in right atrial pressure.
- Closure of the ductus venosus and ductus arteriosus occurs over the next several hours as a result of the contraction of smooth muscles in its wall and increased oxygen tension.
- The release of bradykinin and the immediate drop of prostaglandin E at birth also facilitate the closure of the ductus arteriosus.

The changes which occur between pre- and postnatal circulation are summarized below.

Table II-2-3. Adult Vestiges Derived from the Fetal Circulatory System

Changes After Birth	Remnant in Adult
Closure of right and left umbilical arteries	Medial umbilical ligaments
Closure of the umbilical vein	Ligamentum teres of liver
Closure of ductus venosus	Ligamentum venosum
Closure of foramen ovale	Fossa ovalis
Closure of ductus arteriosus	Ligamentum arteriosum

SEPTATION OF THE HEART TUBE

Except for the **sinus venosus** of the embryonic heart tube that initially develops into right and left horns, the ventricular, atrial, and truncus parts of the heart tube, which are originally a common chamber, will undergo septation into a right and left heart structure. The septation of the atria and ventricles occurs simultaneously beginning in week 4 and is mostly finished in week 8. Most of the common congenital cardiac anomalies result from defects in the formation of these septa.

Atrial Septation

During fetal life, blood is shunted from the right to the left atrium via the foramen ovale (FO). Note that during fetal circulation, right atrial pressure is higher than left due to the large bolus of blood directed into the right atrium from the placenta and to high pulmonary resistance.

The FO has to remain open and functional during the entire fetal life to shunt oxygenated blood from the right atrium into the left atrium.

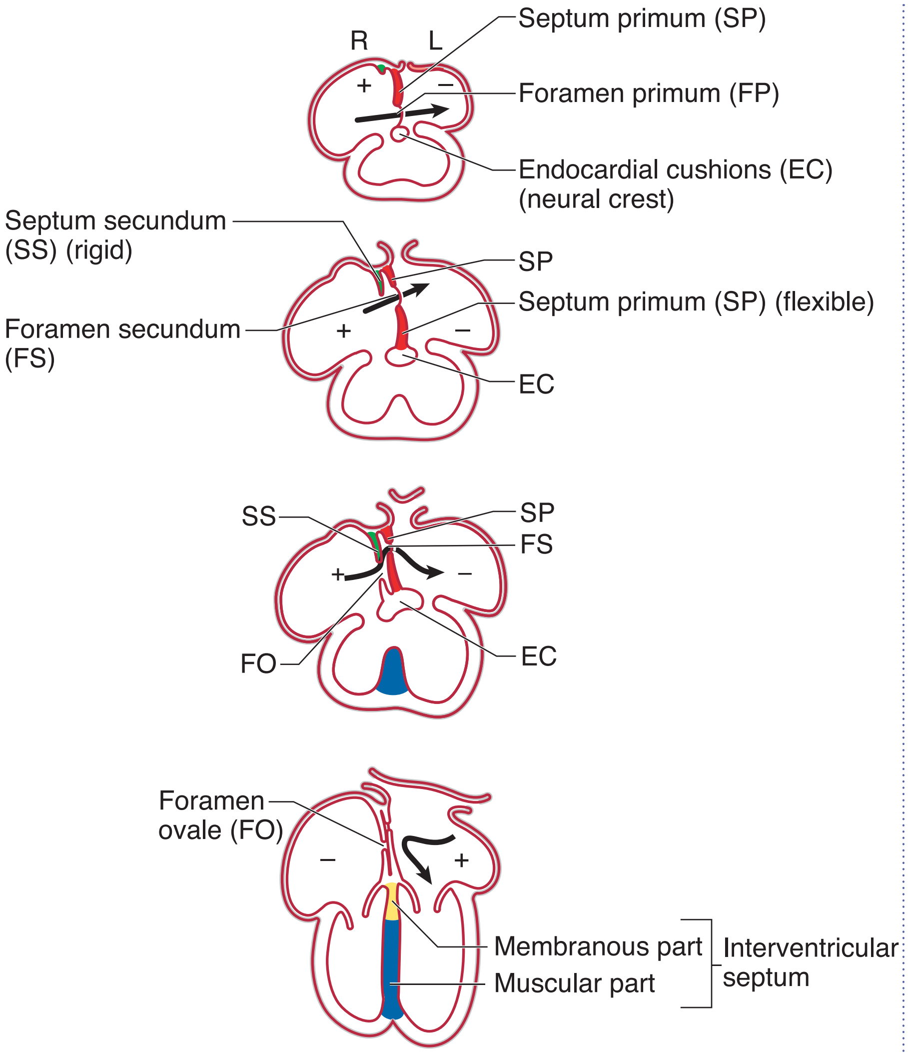

Figure II-2-20. Formation of Atrial Septum

Beginning week 4, the **common atrium** is divided into right and left atria by a series of events involving 2 septa and 2 foramina.

- The flexible **septum primum** (SP) grows inferiorly from the roof of the common atrium toward the **endocardial cushions**, a centrally located mass of mesoderm in the developing heart. Initially, the SP does not reach and fuse with the endocardial cushions. The endocardial cushions form the right and left atrioventricular canals and contribute to the formation of the atrioventricular valves, membranous part of the interventricular septum, and aorticopulmonary septum. **Neural crest cells** migrate into the cushions and facilitate their development.
- The **foramen primum** (FP) is located between the inferior edge of the SP and the endocardial cushion; it is obliterated when the SP later fuses with the endocardial cushions.
- The **foramen secundum** (FS) forms within the upper part of the SP just before the FP closes to maintain the right-to-left shunting of oxygenated blood that entered the right atrium via the inferior vena cava.

- The rigid **septum secundum** (SS) forms to the right of the SP from the roof of the atrium, descends, and partially covers the FS. It does not fuse with the endocardial cushions.
- The **foramen ovale** (FO) is the opening between SP and SS.

Closure of the FO normally occurs immediately after birth; it is caused by increased left atrial pressure resulting from changes in pulmonary circulation and decreased right atrial pressure due to the closure of the umbilical vein.

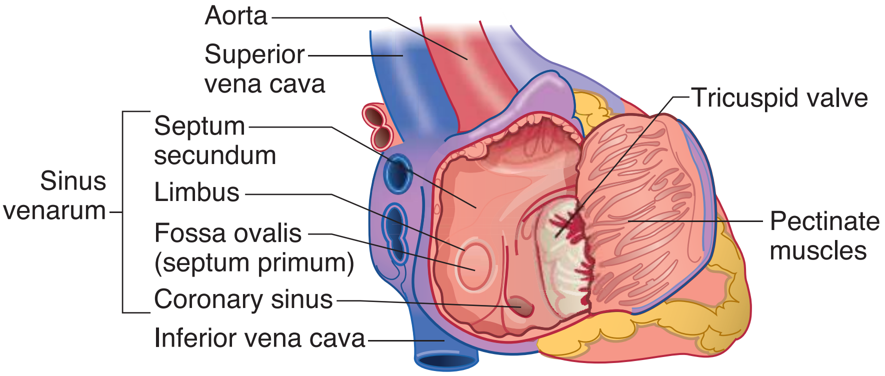

Figure II-2-21. Postnatal Atrial Septum

Atrial Septal Defects

Atrial septal defect (ASD) is one of several congenital heart defects. It is more common in female births than in male. Postnatally, ASDs result **in left-to-right shunting** and are **non-cyanotic conditions**. Two clinically important ASDs are the **secundum** and **primum** types.

- **Secundum-type ASD** is the most common ASD. It is caused by either an excessive resorption of the SP or an underdevelopment and reduced size of the SS or both. This ASD results in variable openings between the right and left atria in the central part of the atrial septum above the limbus. If the ASD is small, clinical symptoms may be delayed as late as age 30.
- **Primum type ASD** is less common than secundum ASD and results from a failure of the septum premium to fuse with the endocardial cushions, and may be combined with defects of the endocardial cushions. Primum ASDs occur in the lower aspect of the atrial wall, usually with a normal formed fossa ovalis. If the endocardial cushion is involved, a primum ASD can also be associated with a defect of the membranous interventricular septum and the atrioventricular valves.

Note

Postnatal Shunts

Right-to-left shunts are **cyanotic conditions**. **Left-to-right** shunts are **non-cyanotic conditions**.

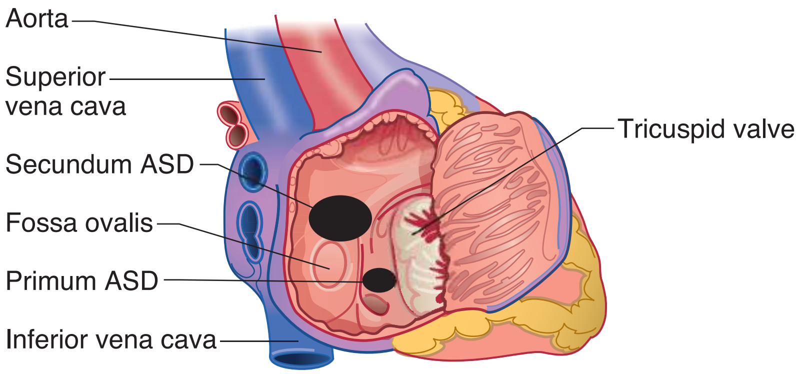

Figure II-2-22. Secundum and Primum Atrial Septal Defect

Ventricular Septation

The development of the **interventricular (IV) septum** begins in week 4 and is usually completed by the end of week 7. Unlike atrial septation, the IV septum will develop and close completely by week 8 without any shunting between the ventricles.

The adult IV septum consists of 2 parts: a large, **muscular component** forming most of the septum and a thin, **membranous part** forming a small component at the superior aspect of the septum.

- The **muscular IV septum** develops in the floor of the ventricle, ascends, and partially separates the right and left ventricles, leaving the **IV foramen**.
- The **membranous IV septum** closes the IV foramen. It forms by the fusion of the right conotruncal ridge, the left conotruncal ridge, endocardial cushion (**neural crest cells** are associated with the endocardial cushion and conotruncal ridges).

Clinical Correlate

Ventricular septal defect (VSD) is the most common of the congenital heart defects, being more common in males than in females. The most common form is a **membranous VSD**, associated with the failure of **neural crest** cells to migrate into the endocardial cushions. A membranous VSD is caused by the failure of the membranous interventricular (IV) septum to develop, and it results in **left-to-right shunting** of blood through the IV foramen.

Patients with left-to-right shunting complain of **excessive fatigue upon exertion**. Left-to-right shunting of blood is **noncyanotic** but causes increased blood flow and pressure to the lungs (pulmonary hypertension). Pulmonary hypertension causes marked proliferation of the tunica intima and media of pulmonary muscular arteries and arterioles. Ultimately, the pulmonary resistance becomes higher than systemic resistance and causes **right-to-left shunting** of blood and late **cyanosis**. At this stage, the condition is called **Eisenmenger complex**.

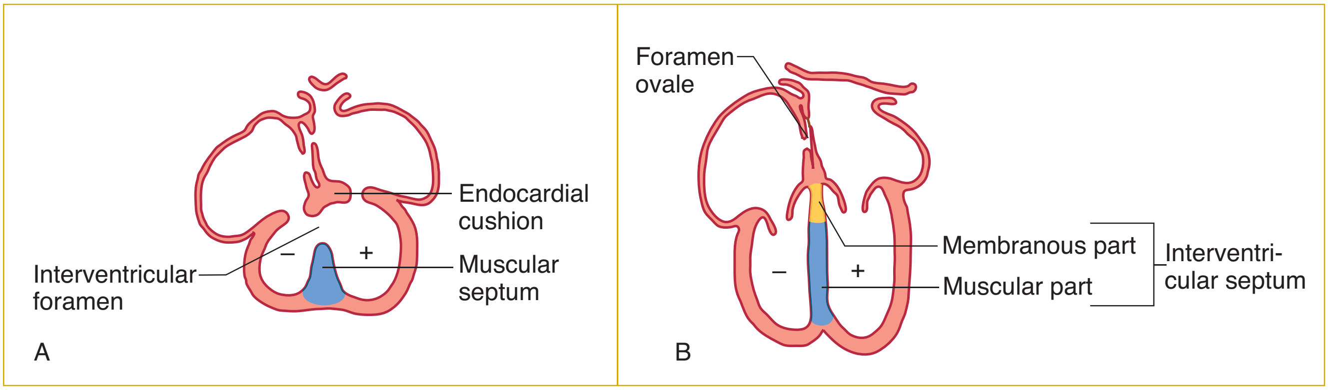

Figure II-2-23. Interventricular Septum

Patent Ductus Arteriosus

A **patent ductus arteriosus** (PDA) occurs when the ductus arteriosus (a connection between the pulmonary trunk and aorta) fails to close after birth. PDA is common in premature infants and in cases of maternal rubella infection.

- Postnatally, a PDA causes a **left-to-right** shunt (from aorta to pulmonary trunk) and is **non-cyanotic**. The newborn presents with a machine-like murmur.
- Normally, the ductus arteriosus closes within a few hours after birth via smooth-muscle contraction to form the **ligamentum arteriosum**. Prostaglandin E (PGE) and low oxygen tension sustain patency of the ductus arteriosus in the fetal period.
- PGE is used to keep the PDA open in certain heart defects (transposition of great vessels).
- PGE inhibitor (e.g., indomethacin), acetylcholine, histamine, and catecholamines promote closure of the ductus arteriosus in a premature birth.

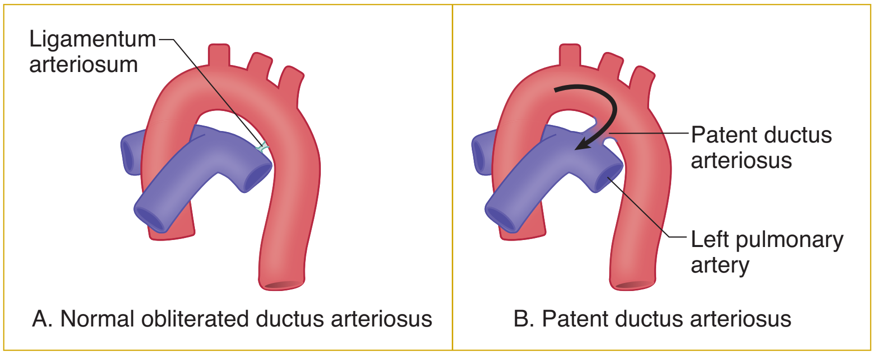

Figure II-2-24. Ductus Arteriosus

Septation of the Truncus Arteriosus

The **septation of the truncus arteriosus** occurs during week 8. **Neural crest cells** migrate into the conotruncal and bulbar ridges of the truncus arteriosus, which grow in a **spiral fashion** and fuse to form the **aorticopulmonary (AP) septum**. The AP septum divides the truncus arteriosus into the **aorta** and **pulmonary trunk**.

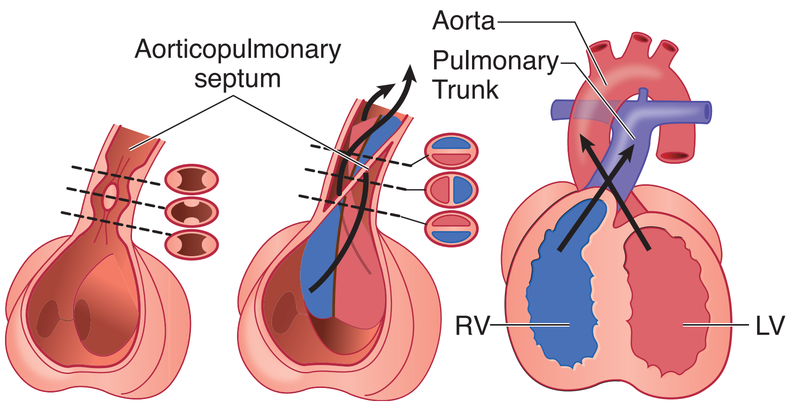

Figure II-2-25. Formation of the Aorticopulmonary Septum

There are 3 classic **cyanotic congenital heart abnormalities** that occur with defects in the development of the aorticopulmonary septum. They are related to the failure of neural crest cells to migrate into the truncus arteriosus:

- **Tetralogy of Fallot (most common)** occurs when the AP septum fails to align properly and shifts anteriorly to the right. This causes **right-to-left** shunting of blood with resultant **cyanosis** that is usually present sometime after birth. Imaging typically shows a boot-shaped heart due to the enlarged right ventricle. There are 4 major defects in tetralogy of Fallot:
  - Pulmonary stenosis (most important)
  - Membranous interventricular septal defect
  - Right ventricular hypertrophy (develops secondarily)
  - Overriding aorta (receives blood from both ventricles)

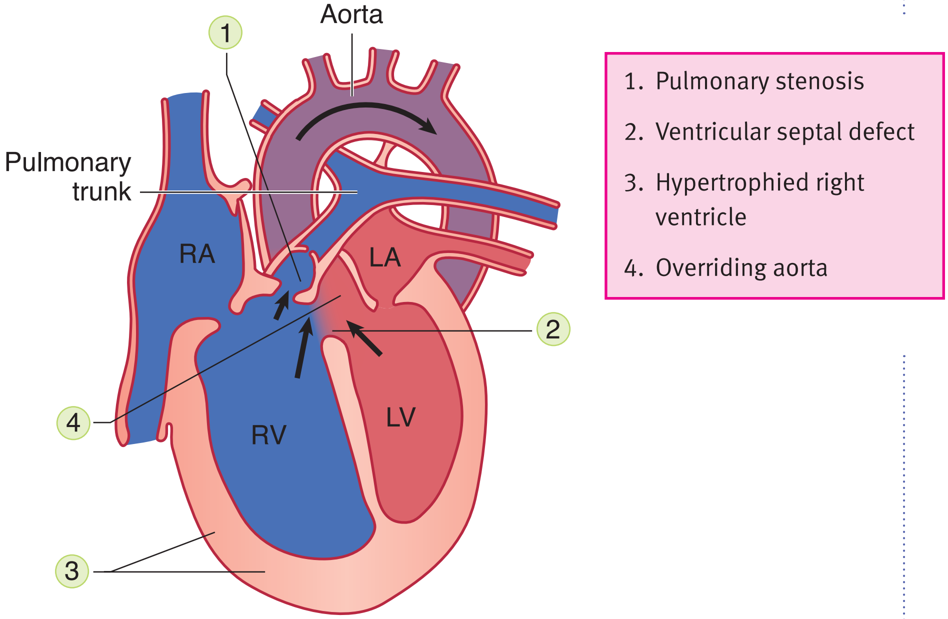

Figure II-2-26. Tetralogy of Fallot

- **Transposition of the great vessels** occurs when the AP septum fails to develop in a spiral fashion and results in the aorta arising from the right ventricle and the pulmonary trunk arising from the left ventricle. This causes **right-to-left s**hunting of blood with resultant cyanosis.
  - Transposition is the most common cause of severe cyanosis that persists immediately at birth. Transposition results in producing 2 closed circulation loops.
  - Infants born alive with this defect usually have other defects (PDA, VSD, ASD) that allow mixing of oxygenated and deoxygenated blood to sustain life.

1. Aorta arises from right ventricle
2. Pulmonary trunk arises from left ventricle
3. Usually associated with a VSD, ASD, or patent ductus arteriosus

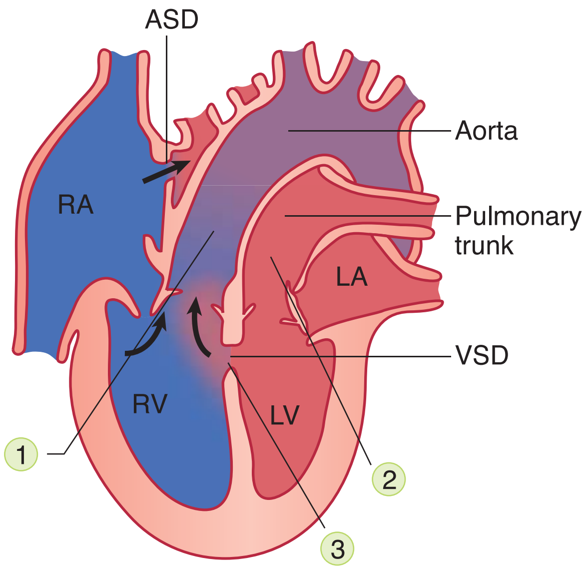

Figure II-2-27. Transposition of the Great Vessels

- Persistent truncus arteriosus occurs when there is only partial development of the AP septum. This results in a condition where only one large vessel leaves the heart that receives blood from both the right and left ventricles**.** This causes **right-to-left** shunting of blood with resultant **cyanosis**. This defect is **always accompanied** by a membranous VSD.

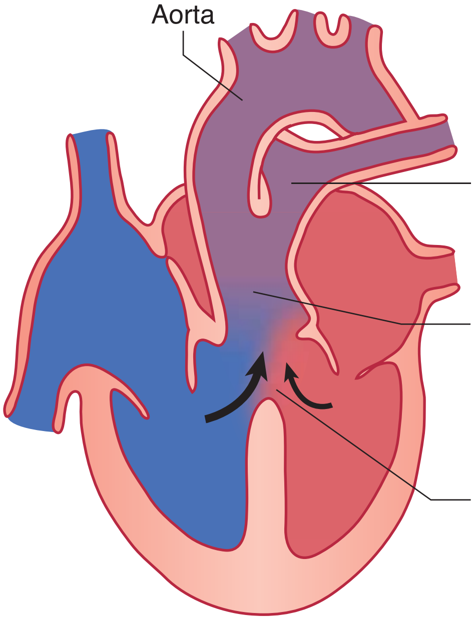

Figure II-2-28. Persistent Truncus Arteriosus

MEDIASTINUM

The **mediastinum** is the central, midline compartment of the thoracic cavity. It is bounded anteriorly by the sternum, posteriorly by the 12 thoracic vertebrae, and laterally by the pleural cavities.

- Superiorly, the mediastinum is continuous with the neck through the **thoracic inlet**; and inferiorly, is closed by the diaphragm. The mediastinum contains most of the viscera of the thoracic cavities except from the lungs (and pleura) and the sympathetic trunk.
- The sympathetic trunks are primarily located paravertebrally, just outside the posterior mediastinum. However, the greater, lesser, and least thoracic splanchnic nerves, which convey preganglionic sympathetic fibers to the collateral (prevertebral) ganglia below the diaphragm, enter the posterior mediastinum after leaving the sympathetic trunks.
- The mediastinum is divided into **superior** and **inferior** mediastina by a plane passing from the **sternal angle (of Louis)** anteriorly to the intervertebral disc between T4 and T5 posteriorly. The sternal angle and plane are **important clinical landmarks**. The inferior mediastinum is classically subdivided into **anterior, middle, and posterior** mediastina.

Note

Non-cyanotic congenital (left-to-right) heart defects at birth:

- Atrial septal defect
- Ventricular septal defects
- Patent ductus arteriosus

Cyanotic congenital (right-to-left) heart defects at birth

- Transposition of great vessels
- Tetralogy of Fallot
- Persistent truncus arteriosus

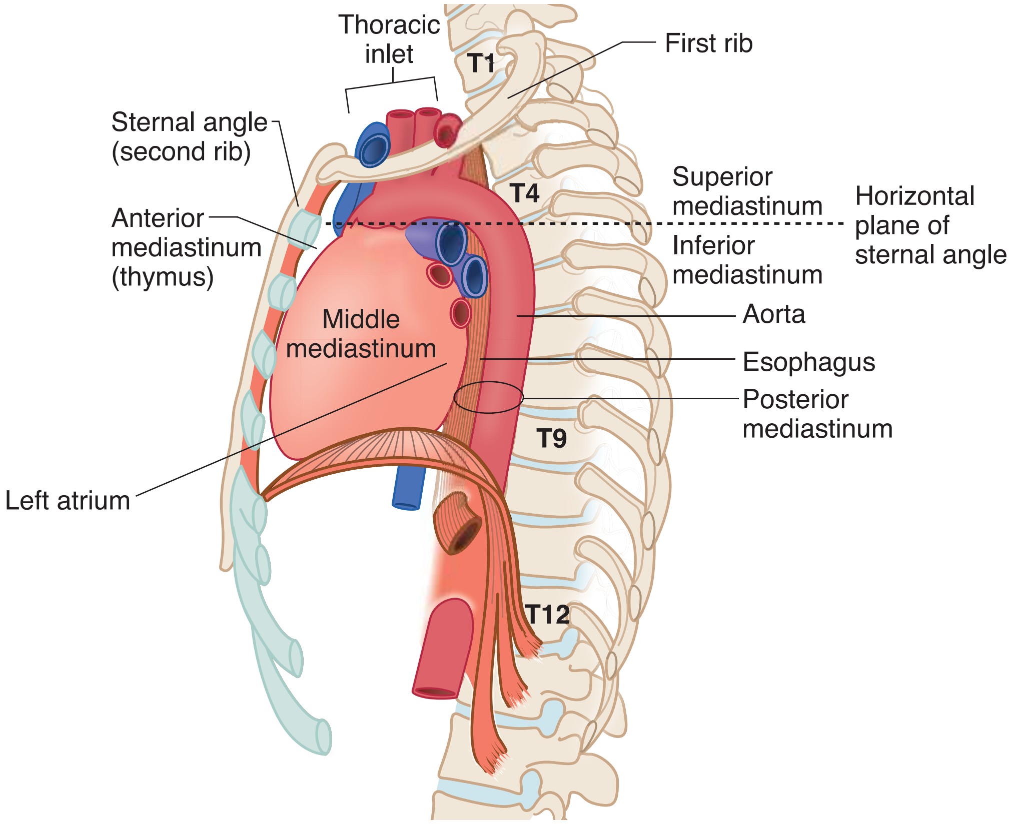

Figure II-2-29. Divisions of the Mediastinum

Anterior Mediastinum

The anterior mediastinum is the small interval between the sternum and the anterior surface of the pericardium. It contains fat and areolar tissue and the inferior part of the **thymus gland**. A tumor of the thymus (**thymoma**) can develop in the anterior or superior mediastinum.

Posterior Mediastinum

The **posterior mediastinum** is located between the posterior surface of the pericardium and the T5-T12 thoracic vertebrae. Inferiorly, it is closed by the diaphragm. There are 4 vertically oriented structures coursing within the posterior mediastinum:

- **Thoracic (descending) aorta**
  - Important branches are the **bronchial, esophageal,** and **posterior intercostal arteries**
  - Passes through the **aortic hiatus** (with the thoracic duct) at the T12 vertebral level to become the abdominal aorta
- **Esophagus**
  - Lies immediately posterior to the left primary bronchus and the **left atrium,** forming an important radiological relationship

  - Covered by the **anterior and posterior esophageal plexuses**, which are derived from the left and right vagus nerves, respectively
  - Passes through the **esophageal hiatus** (with the vagal nerve trunks) at the T10 vertebral level
  - Is constricted (1) at its origin from the pharynx, (2) posterior to the arch of the aorta, (3) posterior to the left primary bronchus, and (4) at the esophageal hiatus of the diaphragm

- **Thoracic duct**
  - Lies posterior to the esophagus and between the thoracic aorta and azygos vein
  - Ascends the posterior and superior mediastina and drains into the junction of the left subclavian and internal jugular veins
  - Arises from the cisterna chyli in the abdomen (at vertebral level L1) and enters the mediastinum through the **aortic hiatus** of the diaphragm

- **Azygos system of veins**
  - Drains the posterior and thoracic lateral wall
  - Communicates with the inferior vena cava in the abdomen and terminates by arching over the root of the right lung to empty into the **superior vena cava** above the pericardium
  - Forms a **collateral venous circulation** between the inferior and superior vena cava

Middle Mediastinum

The middle mediastinum contains the heart and great vessels and pericardium, which will be discussed later.

Superior Mediastinum

The **superior mediastinum** is located between the manubrium of the sternum, anteriorly, and the thoracic vertebrae 1-4, posteriorly. As with all mediastina, the parietal pleura and the lungs form the lateral boundary. The thoracic inlet connects superiorly with the neck and the horizontal plane through the sternal angle forms the inferior boundary.

- The superior mediastinum contains the thymus, great arteries and veins associated with the upper aspect of the heart, trachea, and esophagus.
- The vagus and phrenic nerves and the thoracic duct also course through the mediastinum.
- The pulmonary trunk and arteries are located completely in the middle mediastinum and are not found in the superior mediastinum.

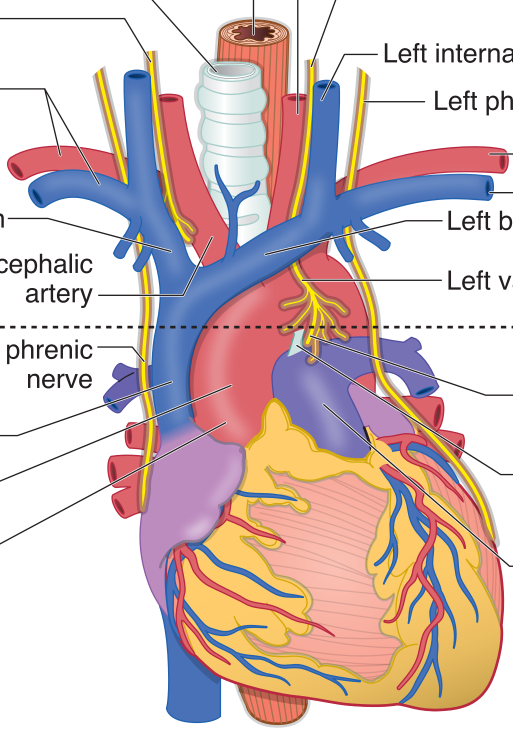

Figure II-2-30. Structures of the Mediastinum

The relationships of these structures in the superior mediastinum are best visualized in **a ventral to dorsal** orientation between the sternum anteriorly and the vertebrae posteriorly:

- **Thymus**: located posterior to the manubrium, usually atrophies in the adult and remains as fatty tissue
- **Right and left brachiocephalic veins**: right vein descends almost vertically and left vein obliquely crosses the superior mediastinum posterior to the thymic remnants
  - The 2 veins join to form the **superior vena cava** posterior to the **right first costal cartilage**.
  - The superior vena cava descends and drains into the right atrium **deep to the right third costal cartilage.**
- **Aortic arch and its 3 branches**: aortic arch begins and ends at the plane of the sternal angle and is located just **inferior** to the left brachiocephalic vein.
  - As a very important radiological landmark, the origins of the 3 branches of the aortic arch (**brachiocephalic, left common carotid, and left subclavian)** are directly **posterior** to the left brachiocephalic vein.

- **Trachea**: lies posterior to the aortic arch and bifurcates at the level of T4 vertebra to form right and left primary bronchi
  - The **carina** is an internal projection of cartilage at the bifurcation.
- **Esophagus**: lies posterior to the trachea and courses posterior to left primary bronchus to enter the posterior mediastinum

ln addition to these structures, the superior mediastinum also contains the **right and left vagus** and **phrenic nerves** and the superior end of the **thoracic duct.**

- Right and left **vagus nerves** contribute to the pulmonary and cardiac plexuses. In the neck, the right vagus nerve gives rise to the **right recurrent laryngeal nerve**, which passes under the right subclavian artery to ascend in the groove between the esophagus and the trachea to reach the larynx. **Note:** The right recurrent laryngeal nerve is not in the mediastinum. The left vagus nerve gives rise to the **left recurrent laryngeal nerve** in the superior mediastinum, which passes under the aortic arch and ligamentum arteriosum to ascend to the larynx.
- The **thoracic duct** is the largest lymphatic channel in the body. It returns lymph to the venous circulation at the junction of the left internal jugular vein and the left subclavian vein.
- **Phrenic nerves** arise from the ventral rami of **cervical nerves 3, 4, and 5.** The nerves are the sole motor supply of the diaphragm and convey sensory information from the central portion of both the superior and inferior portions of the diaphragm and parietal pleura. Both phrenic nerves pass through the middle mediastinum lateral between the fibrous pericardium and pleura, and anterior to the root of the lung.

Clinical Correlate

The **left recurrent laryngeal nerve** (Figure II-2-30) curves under the aortic arch distal to the ligamentum arteriosum where it may be damaged by pathology (e.g., malignancy or aneurysm of the aortic arch), resulting in paralysis of the left vocal folds. The right laryngeal nerve is not affected because it arises from the right vagus nerve in the root of the neck and passes under the subclavian artery.

Either the right or the left recurrent laryngeal nerve may be lesioned with thyroid gland surgery.

Coarctation of the Aorta

Coarctation of the aorta is a narrowing of the aorta distal to the origin of the left subclavian artery. Two types are usually identified based on if the constriction is found proximal or distal to the opening of the ductus arteriosus (DA).

- **Preductal coarctation** (infantile type) is less common and occurs proximal to the DA (Figure II-2-31A). The DA usually remains patent and provides blood flow via the DA to the descending aorta and the lower parts of the body.
- **Postductal coarctation** (adult type) is more common and occurs distal to the DA (Figure II-2-31B). The DA usually closes and obliterates.
  - This results in the intercostal arteries providing collateral circulation between the internal thoracic artery and the thoracic aorta to provide blood supply to the lower parts of the body (Figure II-2-31C).
  - Patients will be hypertensive in the upper body (head, neck, and upper limbs) and hypotensive with weak pulses in the lower limbs.
  - Enlargement of the intercostal arteries results in **costal notching** on the lower border of the ribs, evident in imaging.

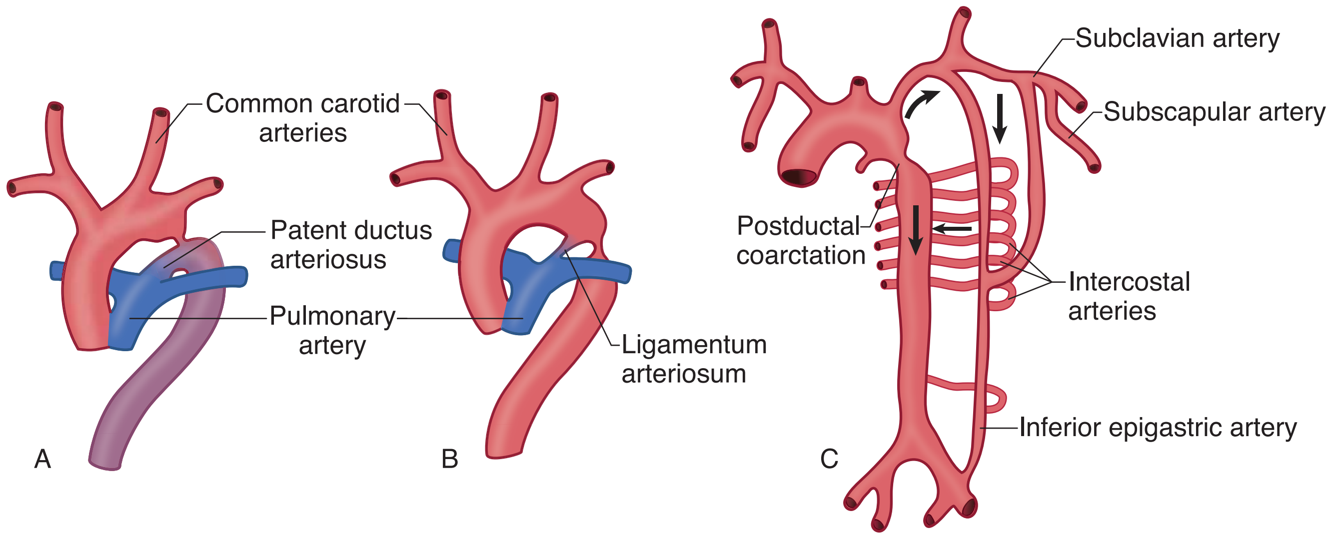

Figure II-2-31. Coarctation of the Aorta: (A) Preductal; (B) Postductal; (C) Collateral Circulation

Middle Mediastinum

The middle mediastinum contains the pericardium, the heart, parts of the great vessels, and the phrenic nerves.

The **pericardium** is the serous sac covering the heart. It is the only one of the serous membranes that has 3 layers: an outer **fibrous layer** and a double-layered **parietal and visceral serous** layers.

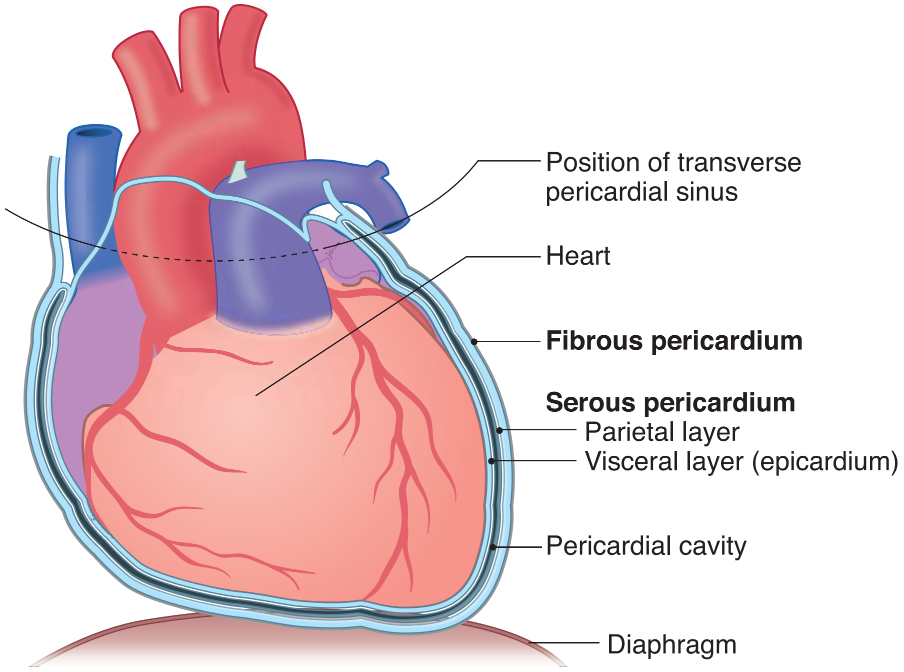

Figure II-2-32. Layers of the Pericardium

The **fibrous pericardium** surrounds the entire heart and the great vessels at the upper aspect of the heart. It is firmly attached below to the central tendon of the diaphragm and superiorly to the adventitia of the great vessels at the plane of the sternal angle (level of the second rib). The fibrous pericardium is very strong and maintains the position of the heart within the middle mediastinum.

The **serous pericardium** is double-layered and formed by the outer **parietal layer** that lines the inner aspect of the fibrous pericardium and the inner **visceral layer** (epicardium) that covers the surface of the heart. The reflection between these 2 serous layers is at the base of the great vessels.

The **pericardial cavity** is the potential space between the parietal and visceral layers containing a small amount of serous fluid that allows free movement of the beating heart. The pericardial cavity is expanded to form 2 sinuses:

- The **transverse pericardial sinus** is a space posterior to the ascending aorta and pulmonary trunk and anterior to the superior vena cava and pulmonary veins. Note that it separates the great arteries from the great veins. The transverse sinus is useful in cardiac surgery to allow isolation of the aorta and pulmonary trunk.
- The **oblique pericardial sinus** is the blind, inverted, U-shaped space posterior to the heart and bounded by reflection of serous pericardium around the 4 pulmonary veins and the inferior vena cava as they enter the heart.

Clinical Correlate

Cardiac tamponade is the pathological accumulation of fluids (serous or blood) within the pericardial cavity. The fluid compresses the heart and restricts venous filling during diastole and reduces cardiac output. To remove the fluid, **pericardiocentesis** is performed with a needle at the **left infrasternal angle** through the cardiac notch of the left lung.

HEART

External Features

The heart lies obliquely within the middle mediastinum, mostly posterior to the sternum. Externally, the heart can be described by its **borders** and **surfaces.**

Borders of the heart

- **The right border** is formed by the **right atrium**.
- The **left border** is mainly formed by the **left ventricle**.
- The **apex** is the tip of the **left ventricle,** and is found in the left fifth intercostal space.
- The superior border is formed by the right and left auricles plus the conus arteriosus of the right ventricle.
- The inferior border is formed at the diaphragm, mostly by the right ventricle.

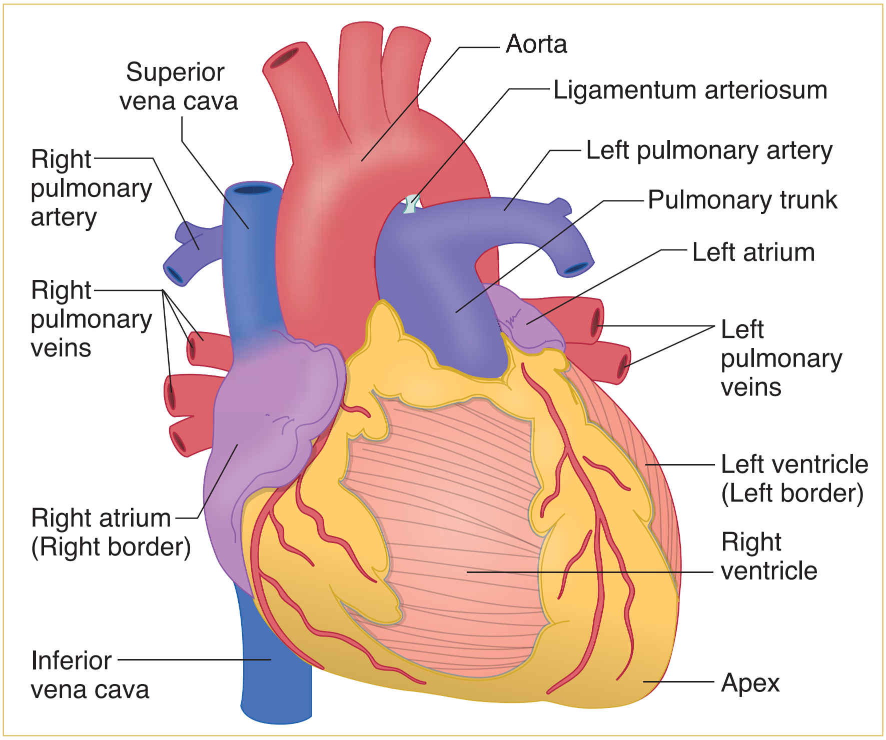

Figure II-2-33. Sternocostal View of the Heart

Surfaces of the heart

- The **anterior (sternocostal) surface** is formed primarily by the **right ventricle**.
- The **posterior surface** is formed primarily by the **left atrium.**
- The **diaphragmatic surface** is formed primarily by the **left ventricle**.

There are 3 main sulci (**coronary** and the **anterior and posterior interventricular**) that course on the surfaces of the heart; they contain the major vessels of the heart and epicardial fat.

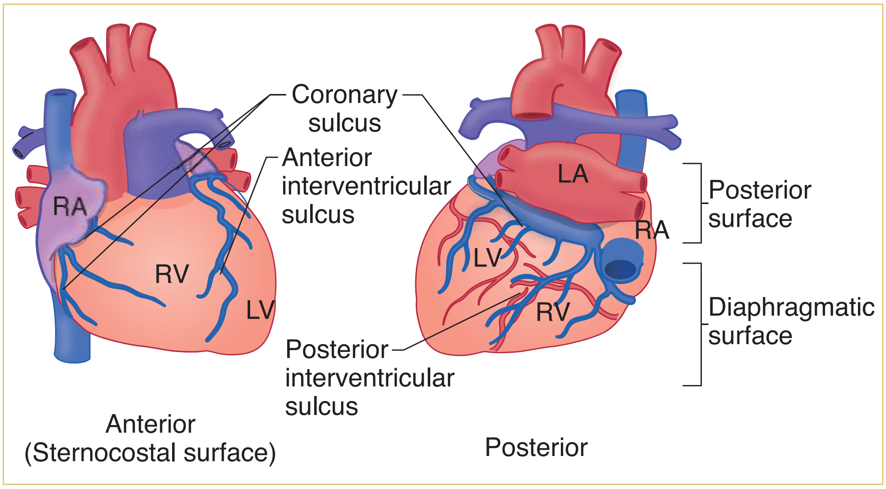

Figure II-2-34. Surfaces of Heart with Sulci

Surface projections

Surface projections of the heart may be traced on the anterior chest wall.

- The **upper right aspect** of the heart is deep to the **third right costal cartilage.**
- The **lower right aspect** of the heart is deep to the **sixth right costal cartilage.**
- The **upper left aspect** of the heart is deep to the **left second costal cartilage**
- The **apex** of the heart is in the **left fifth intercostal space** at the midclavicular line.
- The **right border** extends between the margin of the third right costal cartilage to the sixth right costal cartilage just to the right of the sternum.
- The **left border** extends between the fifth left intercostal space to the second left costal cartilage.
- The **inferior border** extends from the sixth right costal cartilage to the fifth left intercostal space at the midclavicular line.
- The **superior border** extends from the inferior margin of the second left costal cartilage to the superior margin of the third right costal cartilage.

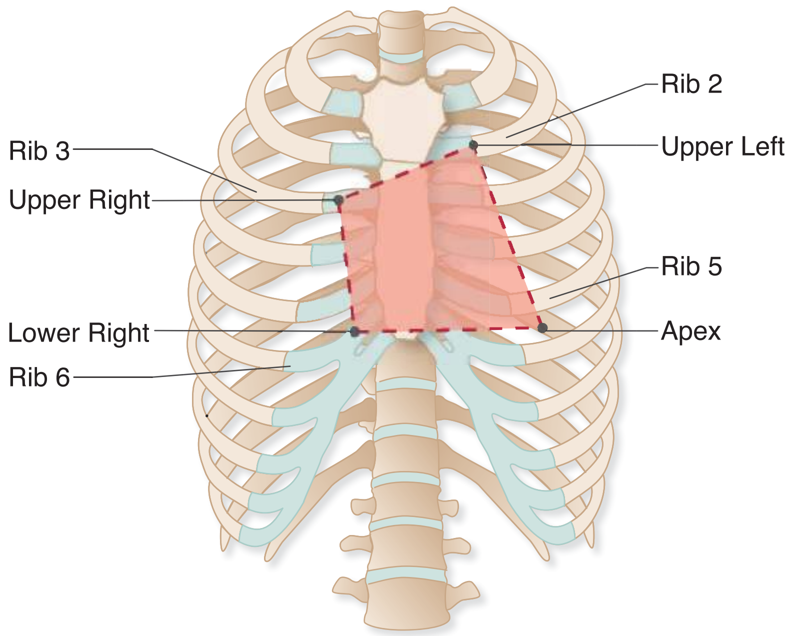

Figure II-2-35. Surface Projections of the Heart

Internal Features

Chambers of the Heart

The **right atrium** receives venous blood from the entire body with the exception of blood from the pulmonary veins.

- The **auricle** is derived from the fetal atrium; it has rough myocardium known as pectinate muscles.
- The **sinus venarum** is the smooth-walled portion of the atrium, which receives blood from the superior and inferior venae cavae. It developed from the **sinus venosus**.
- The **crista terminalis** is the vertical ridge that separates the smooth from the rough portion (pectinate muscles) of the right atrium; it extends longitudinally from the superior vena cava to the inferior vena cava. The **SA node** is in the upper part of the crista terminalis.
- The **fossa ovalis** is close to the foramen ovale, an opening in the interatrial septum which allows blood entering the right atrium from the inferior vena cava to pass directly to the left side of the heart.
- The right AV **(tricuspid) valve** communicates with the right ventricle.

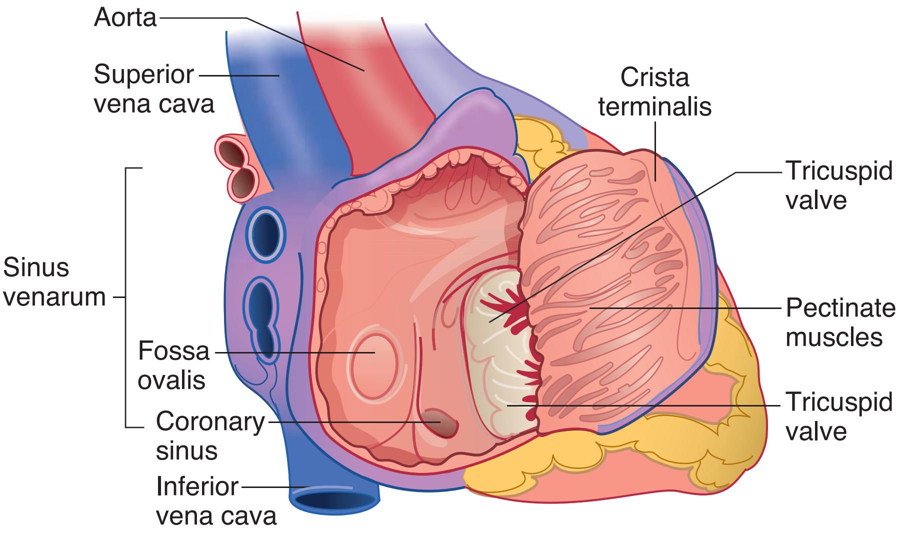

Figure II-2-36. Inside the Right Atrium

The **left atrium** receives oxygenated blood from the lungs via the pulmonary veins. There are 4 openings: upper right and left, and lower right and left pulmonary veins. The left AV orifice is guarded by the mitral **(bicuspid) valve**; it allows oxygenated blood to pass from the left atrium to the left ventricle.

The **right ventricle** receives blood from the right atrium via the tricuspid valve; outflow is to the pulmonary trunk via the pulmonary semilunar valve.

- The **trabeculae carneae** are ridges of myocardium in the ventricular wall.
- The **papillary muscles** project into the cavity of the ventricle and attach to cusps of the AV valve by the strands of the chordae tendineae.

- The **chordae tendineae** are fibrous cords between the papillary muscles and the valve leaflets that control closure of the valve during contraction of the ventricle.
- The **infundibulum** is the smooth area of the right ventricle leading to the pulmonary valve.
- The **septomarginal trabecula (moderator band)** is a band of cardiac muscle between interventricular septum and anterior papillary muscle which conducts part of the cardiac conduction system.

In the **left ventricle**, blood enters from the left atrium through the mitral valve and is pumped out to the aorta through the aortic valve.

- The **trabeculae carneae** are ridges of myocardium in the ventricular wall, normally thicker than those of the right ventricle.
- The **papillary muscles**, usually 2 large ones, are attached by the chordae tendineae to the cusps of the bicuspid valve.
- The **chordae tendineae** act in same way as the right ventricle.
- The **aortic vestibule** leads to the aortic semilunar valve and ascending aorta.

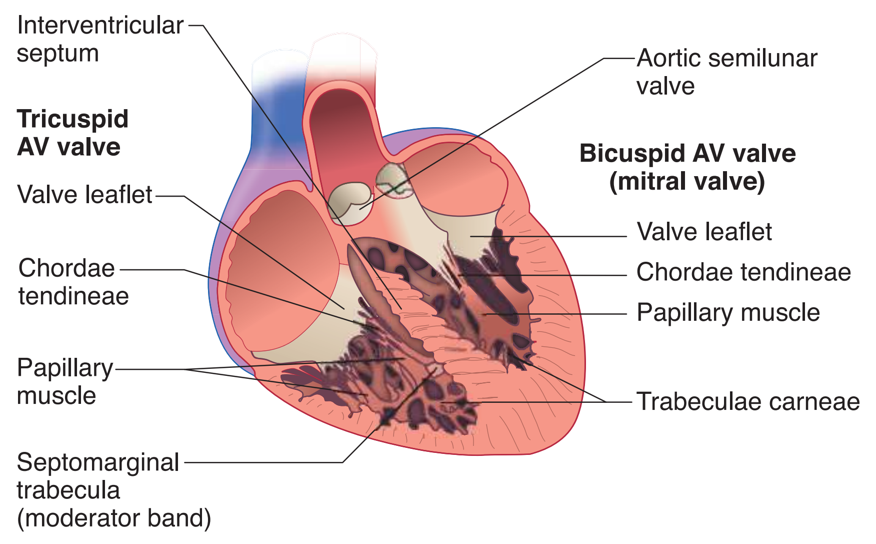

Figure II-2-37. Right and Left Ventricles

HEART HISTOLOGY

Cardiac muscle is striated in the same manner as skeletal muscle, but it differs in being composed of smaller cells (fibers) with only 1 or 2 nuclei. The nuclei are located centrally, instead of peripherally.

Layers of the Heart Wall

The heart wall is composed of 3 distinct layers: an outer epicardium, a middle myocardium and an inner endocardium. The epicardium, or visceral layer of serous pericardium, consists of a simple squamous epithelium (mesothelium) and its underlying connective tissue. The connective tissue contains a large number of fat cells and the coronary vessels. The muscular wall of the heart is the myocardium and is composed mainly of cardiac muscle cells. The endocardium, which lines the chambers of the heart, is composed of a simple squamous epithelium, the endothelium, and a thin layer of connective tissue. Cardiac muscle is striated like skeletal muscle, but these cells are smaller, with centrally placed nuclei. Cardiac muscle has a similar but somewhat less well developed T-tubule system compared to skeletal muscle that is located at the Z-line.

Intercalated Discs

Intercalated discs are special junctional complexes that join myocardial cells. The intercalated discs appear as dark, transverse lines in the light microscope. These disks contain gap junctions and adhering junctions. These junctions permit the spread of electrical (gap) and mechanical (adhering) effects through the walls of the heart, synchronizing activity for the pumping action of the heart chambers. While intercalated discs allow coordinated action of the myocardial cells, the squeezing and twisting movements of the heart chambers (particularly the left ventricle) during systole are due to the disposition of cardiac myocytes.

Purkinje cells are modified cardiac muscle cells with fewer contractile filaments. They are specialized for electrical impulse conduction rather than contraction. Purkinje cells are found in the conduction system of the heart.

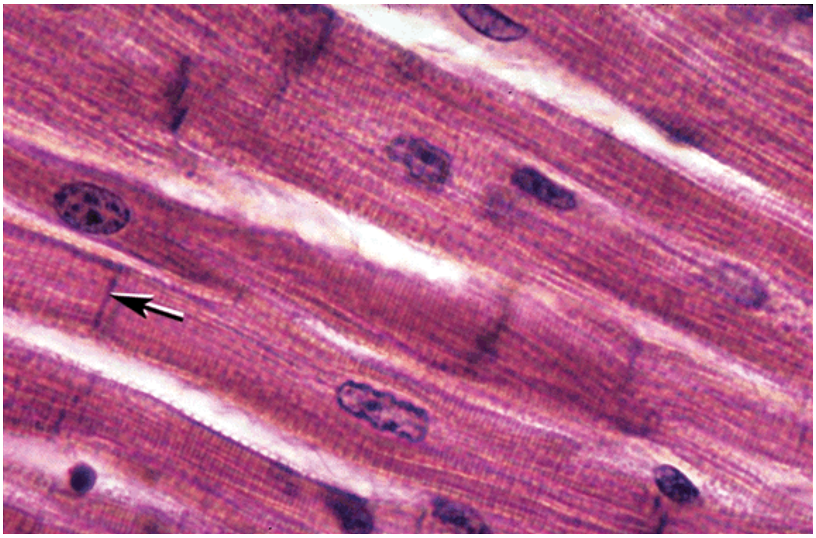

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Figure II-2-38. Cardiac muscle cells with centrally placed nuclei and intercalated disks (arrow)

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Figure II-2-39. EM of part of an intercalated disk with gap junction (arrow) adjacent to an adhering junction with intracellular density.

Auscultation of Heart Valves

Points of auscultation of the **semilunar valves** (aortic and pulmonary) and the **atrioventricular valves** (tricuspid and mitral or bicuspid) are shown below. The first heart sound occurs at the closure of the atrioventricular valves at the beginning of systole and the second heart sound occurs at the closure of the aortic and pulmonary semilunar valves at the end of systole.

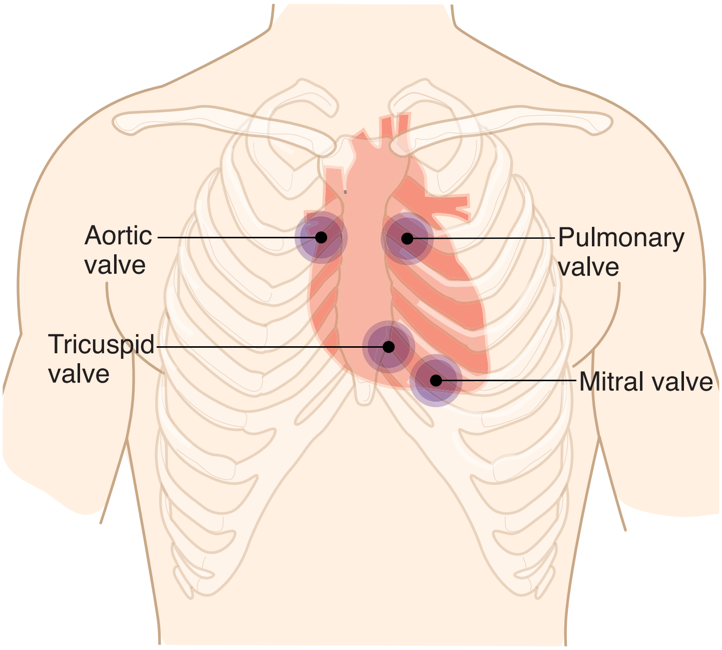

Figure II-2-40. Surface Projections of the Heart

Heart Murmurs

Murmurs in valvular heart disease result when there is **valvular insufficiency or regurgitation** (the valves fail to close completely) or **stenosis** (narrowing of the valves). The **aortic and mitral valves** are more commonly involved in valvular heart disease.

Valves of the Heart

- **Atrioventricular**
  Right heart: tricuspid
  Left heart: bicuspid
- **Semilunar**
  Aortic (3 cusps)
  Pulmonary (3 cusps)

For most of ventricular systole, the mitral valve should be closed and the aortic valve should be open, so that "common **systolic** valvular defects" include **mitral insufficiency** and **aortic stenosis**. For most of ventricular diastole, the mitral valve should be open and the aortic valve should be closed, so that "common **diastolic** valvular defects" include **mitral stenosis** and **aortic insufficiency**.

A heart murmur is heard downstream from the valve. Thus, stenosis is orthograde direction from valve and insufficiency is retrograde direction from valve.

Auscultation sites for mitral and aortic murmurs are shown in Figure II-2-41.

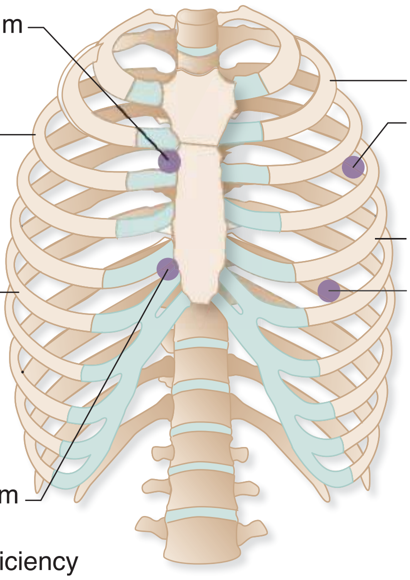

Figure II-2-41. Auscultation of Heart Murmurs

Table II-2-4. Heart Murmurs

	Stenosis	Insufficiency
A-V Valves Tricuspid (right) Mitral (left)	Diastolic murmur	Systolic murmur
Outflow Valves Pulmonic (right) Aortic (left)	Systolic murmur	Diastolic murmur

Arterial Supply of the Heart

The blood supply to the myocardium is provided by branches of the **right and left coronary arteries**. These 2 arteries are the only branches of the ascending aorta and arise from the **right and left aortic sinuses of the ascending aorta**, respectively. Blood flow enters the coronary arteries during diastole.

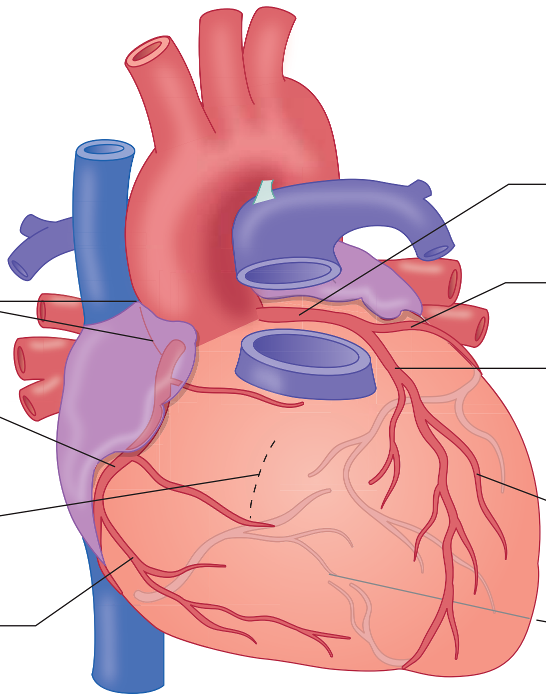

Figure II-2-42. Arterial Supply to the Heart

Right coronary artery

The **right coronary artery** courses in the coronary sulcus and supplies major parts of the right atrium and the right ventricle. The branches include the following:

- **Sinoatrial (SA) nodal artery:** One of the first branches of the right coronary, it encircles the base of the superior vena cava to supply the **SA node**.
- **Atrioventricular (AV) nodal artery:** It arises from the distal end of the right coronary artery as it forms the posterior interventricular artery and penetrates the interatrial septum to supply the **AV node**.
- **Posterior interventricular artery:** It is the terminal distribution of the right coronary artery and courses in the posterior interventricular sulcus to supply parts of the right and left ventricles and, importantly, **the posterior third** of the interventricular septum.

Left coronary artery

The **left coronary artery** travels a short course between the left auricle and ventricle, and divides into 2 branches: **anterior interventricular or left anterior descending (LAD) artery** and **circumflex artery**.

- The **anterior interventricular artery** descends in the anterior interventricular sulcus and provides branches to the (1) **anterior left ventricle wall**, (2) **anterior two-thirds of the interventricular septum**, (3) **bundle of His**, and (4) **apex**. The LAD is the most common site of coronary occlusion.

Clinical Correlate

In myocardial infarction, the left anterior descending artery is obstructed in 50% of cases, the right coronary in 30%, and the circumflex artery in 20% of cases.

- The **circumflex artery** courses around the left border of the heart in the coronary sulcus and supplies (1) the **left border of the heart** via the **marginal branch** and (2) ends on the posterior aspect of the left ventricle and supplies the **posterior-inferior** left ventricular wall.

Venous Drainage of the Heart

The major cardiac veins draining the heart course in the sulci and accompany the arteries but do not carry the same names. The major veins are the following:

- **Coronary sinus** is the main vein of the coronary circulation; it lies in the posterior coronary sulcus and drains to an opening in the right atrium. It develops from the **left sinus venosus**.
- **Great cardiac vein** lies in the anterior interventricular sulcus with the LAD artery; it is the main tributary of the coronary sinus.
- **Middle cardiac vein** lies in the posterior interventricular sulcus with the posterior interventricular artery; it joins the coronary sinus.
- **Venae cordis minimae (thebesian veins) and anterior cardiac veins** open directly to the chambers of the heart.

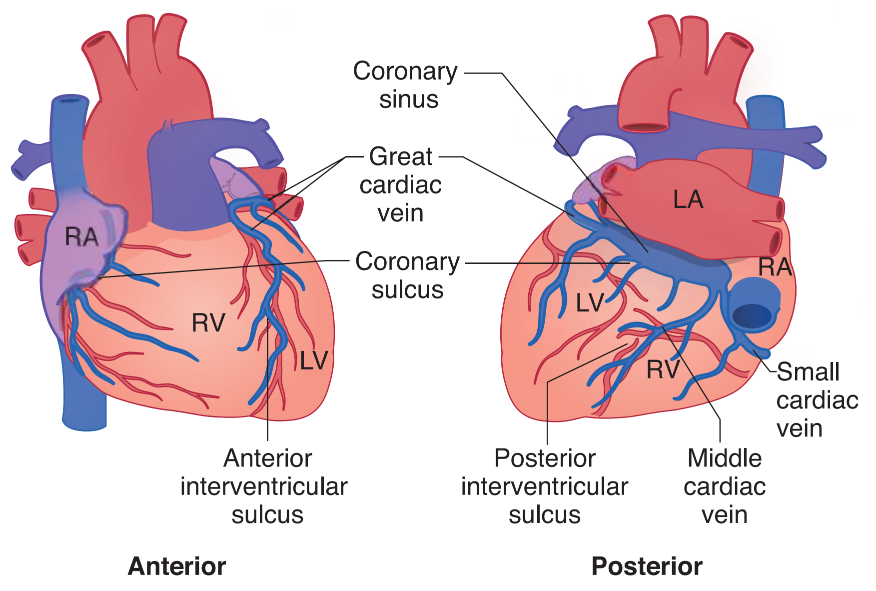

Figure II-2-43. Venous Drainage of the Heart

Conducting System of the Heart

The **cardiac conduction system** is a specialized group of myocardial cells that initiates the periodic contractions of the heart due to their ability to depolarize at a faster rate than other cardiac myocytes. Electrical activity spreads through the walls of the atria from the SA node and is quickly passed by way of internodal fibers to the atrioventricular node. From the atrioventricular node, activity passes through the bundle of His and then down the right and left bundle branches in the interventricular septum. The bundle branches reach additional

specialized cardiac muscle fibers known as Purkinje fibers in the ventricular walls.

- The Purkinje fibers run in several bundles along the endocardial surface and initiate ventricle activity starting at the apex of the ventricles.
- Purkinje fibers have a large cross section, a cytoplasm with few contractile fibrils and a large content of glycogen.

The **SA node** initiates the impulse for contraction of heart muscle (and is therefore termed the "pacemaker" of the heart). It is located at the superior end of the crista terminalis, where the superior vena cava enters the right atrium.

- The SA node is supplied by the SA nodal branch of the **right coronary artery**.
- Impulse production is speeded up by sympathetic nervous stimulation; it is slowed by parasympathetic (vagal) stimulation.

The **AV node** receives impulses from the SA node; it is located in the interatrial septum near the opening of the coronary sinus. The AV node slows the impulse so that it reaches the ventricles after it has reached the atria.

- The AV node is supplied by the **right coronary artery**.

The **bundle of His** originates in the AV node. It conducts impulses to the right and left ventricles. It is supplied by the **LAD artery**.

- In the right ventricle, the moderator band (septomarginal trabecula) contains the right bundle branch.
- Impulses pass from the right and left bundle branches to the papillary muscles and ventricular myocardium.

Innervation

The cardiac plexus is a combination of sympathetic and parasympathetic (vagal) fibers.

- **Sympathetic** stimulation increases the heart rate. Nerves that sense **pain** associated with coronary artery ischemia (angina) follow the **sympathetic pathways** back into spinal cord segments T1–T5.
- **Parasympathetic** stimulation slows the heart rate. Sensory nerves that carry the afferent limb of cardiac **reflexes** travel with the **vagus nerve**.

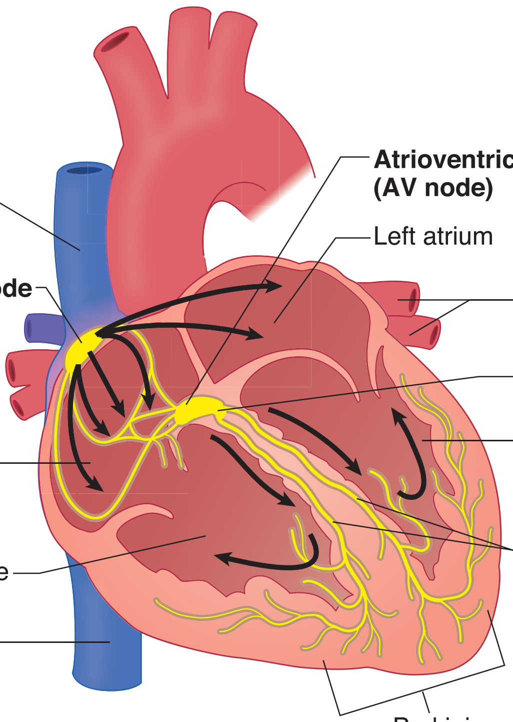

Figure II-2-44. Cardiac Conduction System

DIAPHRAGM

The diaphragm is composed of a muscular portion and a central tendon. It is dome-shaped, and descends upon contraction of its muscular portion. It is innervated by the **phrenic nerves** that arise from spinal cord segments **C3 through C5**.

The diaphragm is formed by the fusion of tissue from 4 sources:

- The septum transversum gives rise to the central tendon of the diaphragm.
- The pleuroperitoneal membranes give rise to parts of the tendinous portion of the diaphragm.
- The dorsal mesentery of the esophagus gives rise to the crura of the diaphragm.
- The body wall contributes muscle to the periphery of the diaphragm.

Apertures in the Diaphragm

Caval hiatus is located to the right of the midline at the level of T8, within the central tendon. It transmits the inferior vena cava and some branches of the right phrenic nerve.

Esophageal hiatus is located to the left of the midline at the level of T10, within the muscle of the right crus. It transmits the esophagus and the anterior and posterior vagus trunks.

Aortic hiatus is located in the midline at the level of T12, behind the 2 crura. It transmits the aorta and thoracic duct.

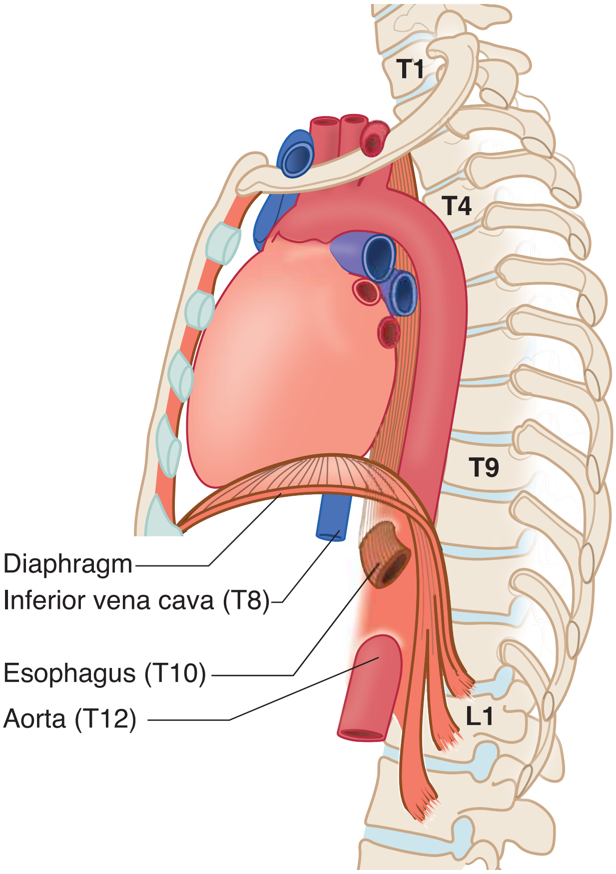

Figure II-2-45. The Diaphragm

Clinical Correlate

Pain Referral

Because the innervation to the diaphragm (motor and sensory) is primarily from C3 through C5 spinal nerves, pain arising from the diaphragm (e.g., subphrenic access) is referred to these dermatomes in the shoulder region.

Clinical Correlate

A **congenital diaphragmatic hernia** is a herniation of abdominal contents into the pleural cavity due to the failure of the **pleuroperitoneal membranes** to develop properly. The hernia is most commonly found on the left posterolateral side and causes pulmonary hypoplasia.

An **esophageal hiatal hernia** is a herniation of the stomach into the pleural cavity due to an abnormally large esophageal hiatus to the diaphragm. This condition renders the esophagogastric sphincter incompetent so that contents reflux into the esophagus.

RADIOLOGY

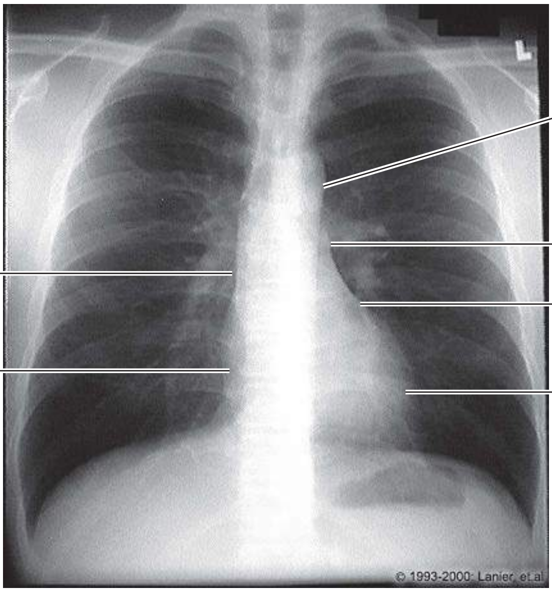

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Figure II-2-46. Anterior Projection of Chest, Male

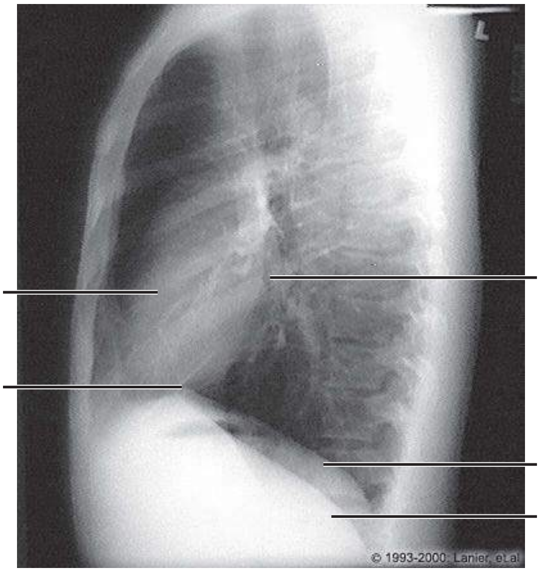

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Figure II-2-47. Lateral Projection of Chest, Male

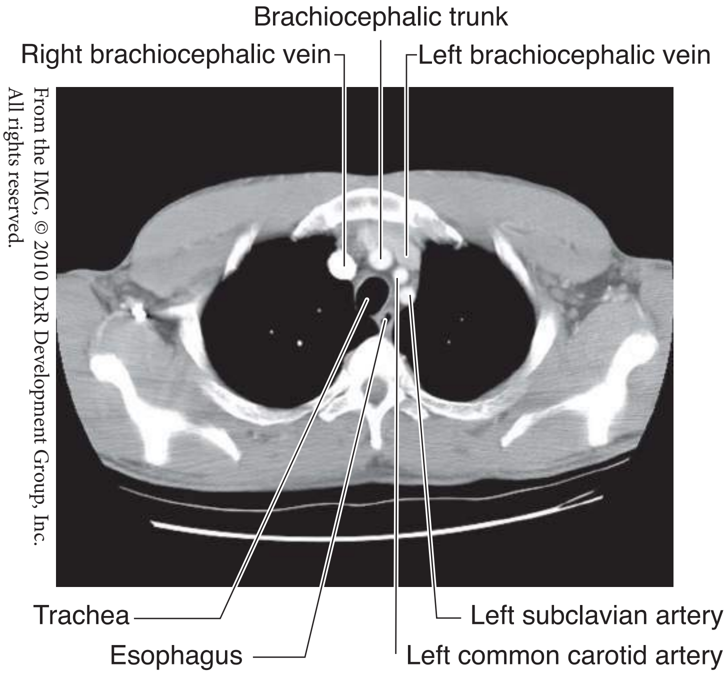

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Figure II-2-48. Chest: CT, T2

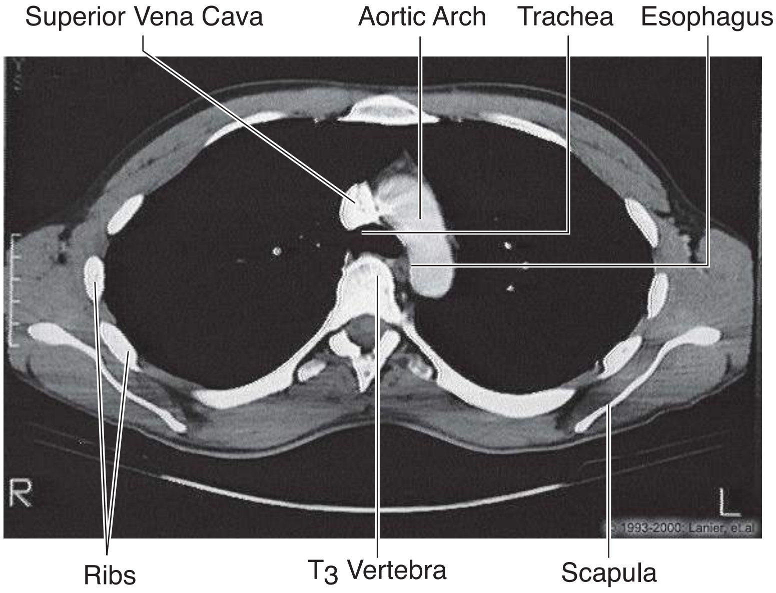

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Figure II-2-49. Chest: CT, T3

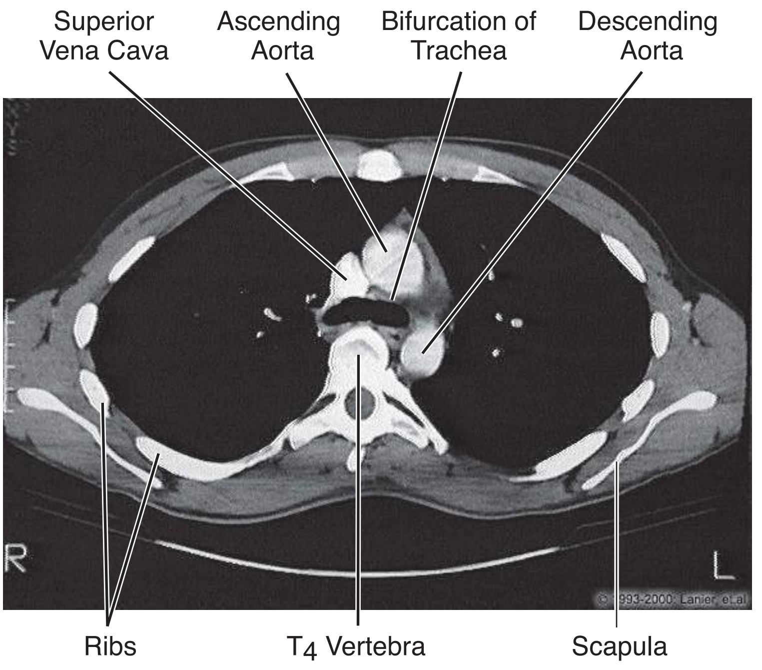

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Figure II-2-50. Chest: CT, T4

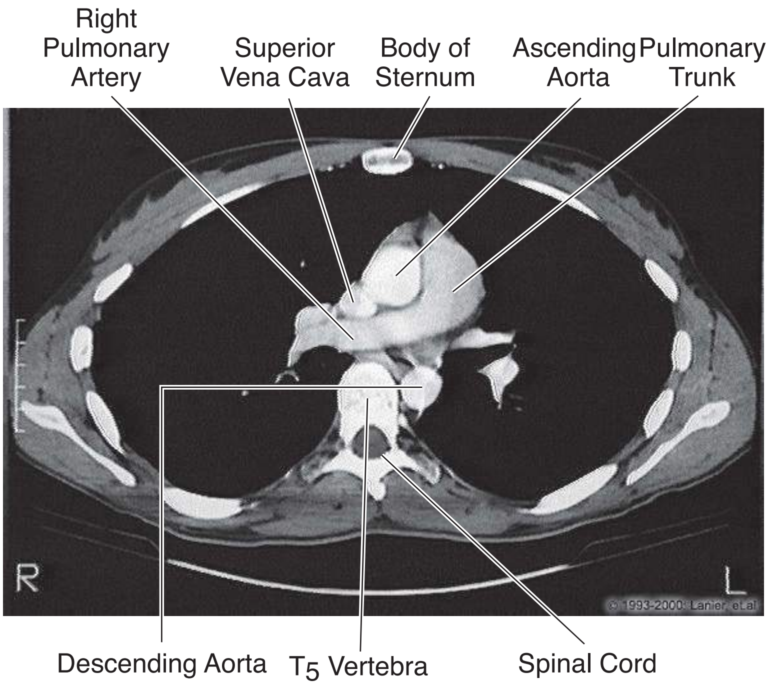

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Figure II-2-51. Chest: CT, T5

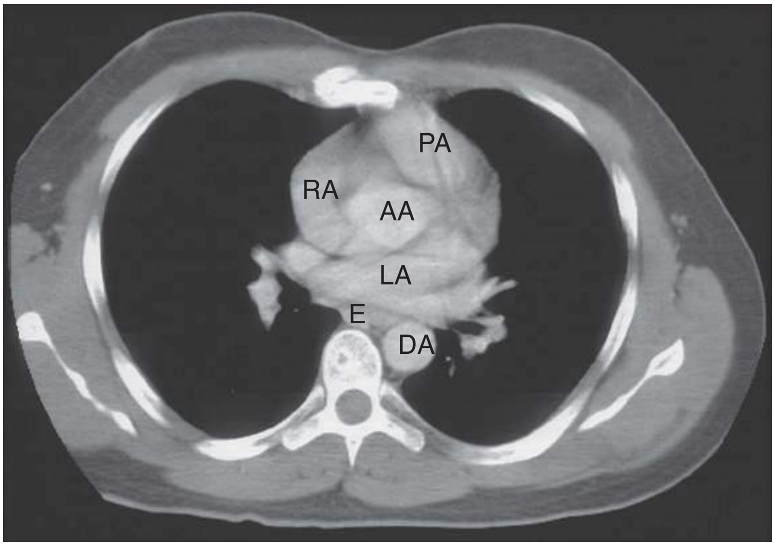

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Figure II-2-52. Chest: CT, T5

PA = pulmonary artery

RA = right atrium

AA = ascending aorta

LA = left atrium

E = esophagus

DA = descending aorta

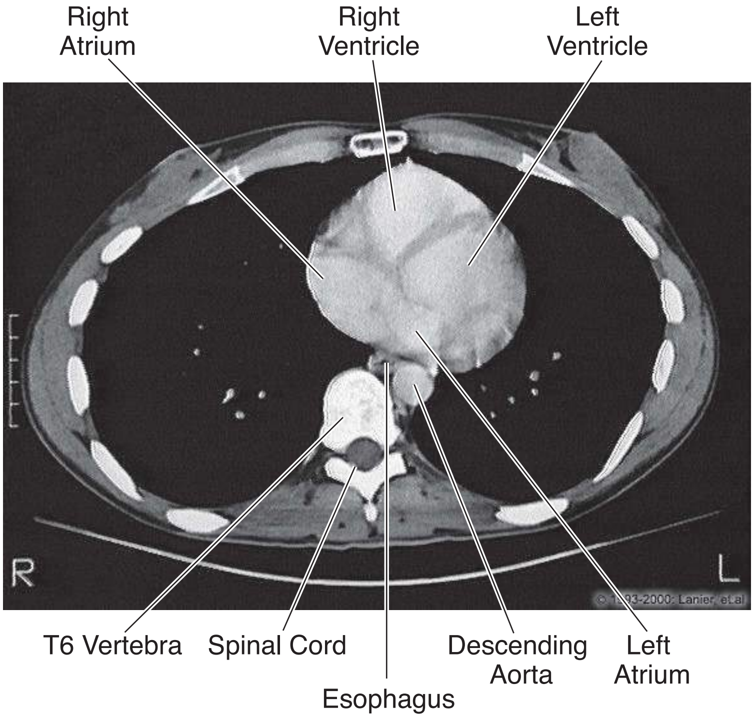

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Figure II-2-53. Chest: CT, T6

Abdomen, Pelvis, and Perineum

3

Learning Objectives

- ❑ Explain information related to inguinal region and canal
- ❑ Answer questions about embryology of the GI system
- ❑ Solve problems concerning peritoneum
- ❑ Answer questions about GI histology, innervation, and immune functions
- ❑ Solve problems concerning arterial supply and venous drainage to abdominal viscera
- ❑ Explain information related to posterior abdominal body wall
- ❑ Answer questions about urinary histology and function
- ❑ Use knowledge of male and female reproductive histology
- ❑ Demonstrate understanding of radiology of the abdomen and pelvis

ANTERIOR ABDOMINAL WALL

Surface Anatomy

The **linea alba** is a shallow groove that runs vertically in the median plane from the xiphoid to the pubis. It separates the right and left rectus abdominis muscles. The components of the rectus sheath intersect at the linea alba.

The **linea semilunaris** is a curved line defining the lateral border of the rectus abdominis, a bilateral feature.

Planes and Regions

The anterior abdominal wall is divided into 9 regions separated by several planes and lines.

The **subcostal plane** (horizontal) passes through the inferior margins of the 10th costal cartilages at the level of the third lumbar vertebra.

The **transpyloric plane** passes through the L1 vertebra, being half the distance between the pubis and the jugular notch. The plane passes through several important abdominal landmarks useful for radiology: pylorus of the stomach (variable), fundus of gallbladder, neck and body of the pancreas, hila of kidneys, first part of the duodenum, and origin of the superior mesenteric artery.

The **midclavicular lines** (vertical) are the 2 planes that pass from the midpoint of the clavicle to the midpoint of the inguinal ligament on each side.

RH: right hypochondrium

LH: left hypochondrium

RL: right lumbar

LL: left lumbar

RI: right inguinal

LI: left inguinal

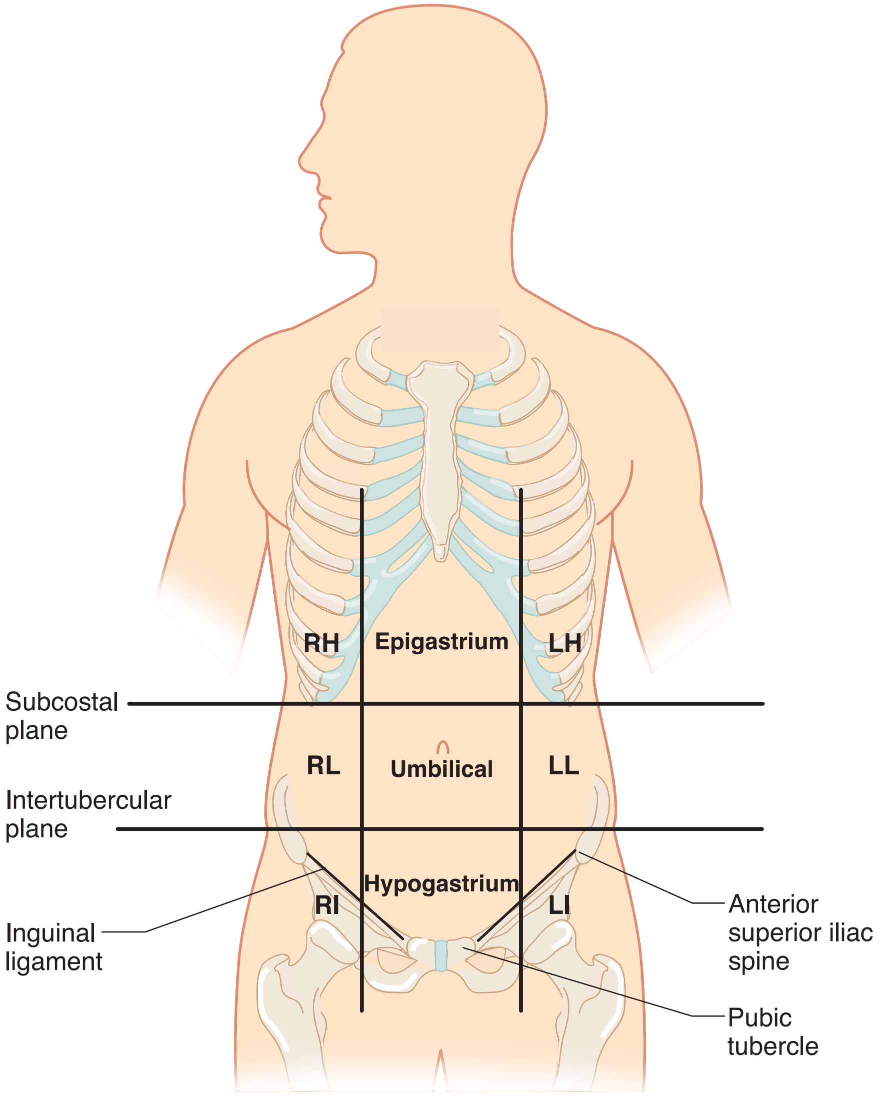

Figure II-3-1. Regions and Planes of the Abdomen

MUSCLES AND FASCIAE OF ANTERIOR BODY WALL

The anterolateral abdominal body wall is a multilayer of fat, fasciae, and muscles (with their aponeuroses) that support and protect the abdominal contents. Three flat abdominal muscles are arranged in layers and the rectus abdominis is oriented vertically adjacent to the midline, extending between the costal margin and the pubis. Abdominal muscles are important in respiration, defecation, micturition, childbirth, etc.

Layers of Anterior Abdominal Wall

The layers of tissue that make up the abdominal wall are described superficial to deep.

Skin

Superficial fascia of the anterior abdominal wall below the umbilicus consists of 2 layers:

- **Camper** (fatty) fascia is the outer, subcutaneous layer of superficial fascia that is variable in thickness owing to the presence of fat.
- **Scarpa** (membranous) fascia is the deeper layer of superficial fascia devoid of fat. It is continuous into the perineum with various perineal fascial layers (Colles' fascia, dartos fascia of the scrotum, superficial fascia of the clitoris or penis).

Muscles

The **external abdominal oblique muscle and aponeurosis** is the most superficial of the 3 flat muscles of the abdominal wall. Its contributions to the abdominal wall and inguinal region are the following:

- **Inguinal ligament** is the inferior rolled under aponeurotic fibers of the external oblique that extend between the anterior superior iliac spine and the pubic tubercle. Medially, the fibers of the inguinal ligament form a flattened horizontal shelf called the **lacunar ligament** that attaches deeply to the pectineal line of the pubis and continues as the pectineal ligament. Lacunar ligament forms the medial border of a femoral hernia.
- **Superficial inguinal ring** is a vertical triangular cleft in the external oblique aponeurosis that represents the medial opening of the inguinal canal just superior and lateral to the pubic tubercle. It transmits the structures of the female and male inguinal canals.
- **External spermatic fascia** is the outer layer of the 3 coverings of the spermatic cord formed at the superficial inguinal ring in males.
- **Rectus sheath**: The external aponeuroses contribute to the anterior layer of the rectus sheath.

Note

Anterior Abdominal Wall Layers

- Skin
- Superficial fascia
  - Camper (fatty)
  - Scarpa (fibrous)
- External oblique
- Internal oblique
- Transversus abdominis
- Transversalis fascia
- Extraperitoneal connective tissue
- Parietal peritoneum

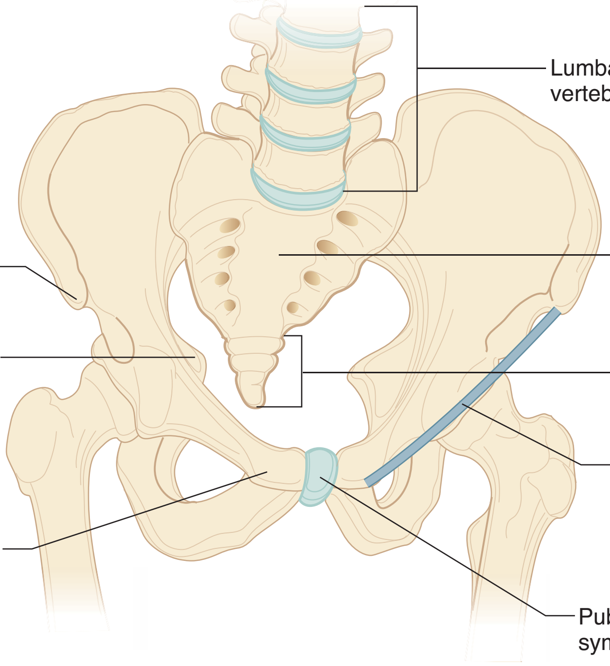

Figure II-3-2. Osteology of the Abdominopelvic Cavity

Internal abdominal oblique muscle and aponeurosis: This middle layer of the 3 flat muscles originates, in part, from the lateral two-thirds of the inguinal ligament. The internal oblique fibers course medially and arch over the inguinal canal in parallel with the arching fibers of the transversus abdominis muscle. The contributions of the internal abdominal oblique to the abdominal wall and inguinal region are the following:

- **Conjoint tendon (falx inguinalis**) is formed by the combined arching fibers of the internal oblique and the transversus abdominis muscles that insert on the pubic crest posterior to the superficial inguinal ring.
- **Rectus sheath:** The internal aponeuroses contribute to the layers of the rectus sheath.
- **Cremasteric muscle and fascia** represent the middle layer of the spermatic fascia covering the spermatic cord and testis in the male. It forms in the inguinal canal.

Transversus abdominis muscle and aponeurosis: This is the deepest of the flat muscles. The transversus muscle originates, in part, from the lateral one-third of the inguinal ligament and arches over the inguinal canal with the internal oblique fibers to contribute to the **conjoint tendon**. The aponeuroses of the transversus muscle also contribute to the layers of the rectus sheath. It does not contribute to any of the layers of the spermatic fasciae.

Abdominopelvic Fasciae and Peritoneum

Transversalis fascia: This fascia forms a continuous lining of the entire abdominopelvic cavity. Its contributions to the inguinal region include the following:

- **Deep inguinal ring** is formed by an outpouching of the transversalis fascia immediately above the midpoint of the inguinal ligament and represents the lateral and deep opening of the inguinal canal. The **inferior epigastric vessels** are **medial** to the deep ring.
- **Internal spermatic fascia** is the deepest of the coverings of the spermatic cord formed at the deep ring in the male.
- **Femoral sheath** is an inferior extension of the transversalis fascia deep to the inguinal ligament into the thigh containing the femoral artery and vein and the femoral canal (site of femoral hernia).
- **Rectus sheath**: The transversalis fascia contributes to the posterior layer of the rectus sheath.

Extraperitoneal connective tissue is a thin layer of loose connective tissue and fat surrounding the abdominopelvic cavity, most prominent around the kidneys. The **gonads** develop from the urogenital ridge within this layer.

Parietal peritoneum is the outer serous membrane that lines the abdominopelvic cavity.

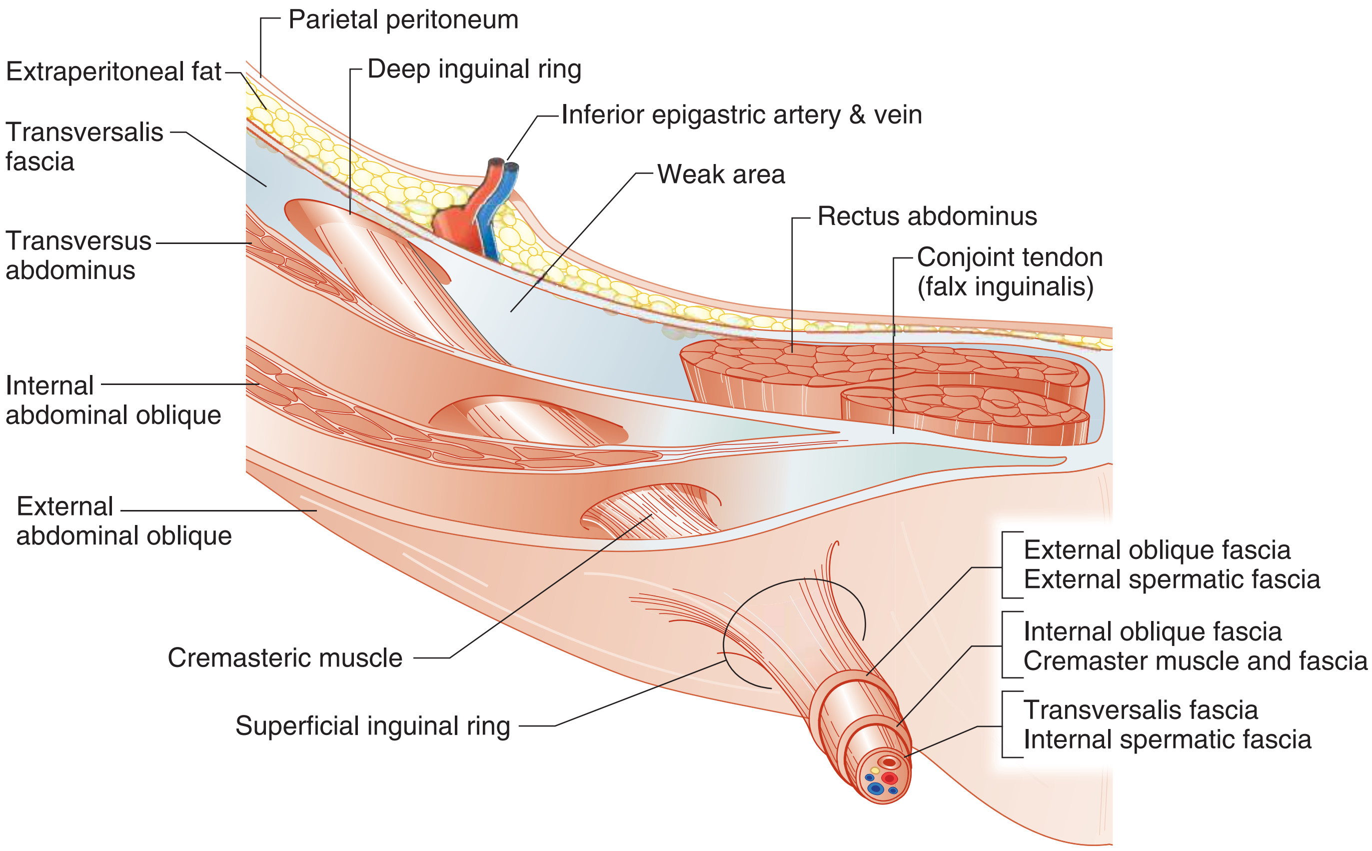

Figure II-3-3. Layers of Anterolateral Abdominal Wall and Inguinal Canal

Nerves, Blood Vessels, and Lymphatics of Abdominal Wall

Innervation of the skin and musculature of the anterior abdominal wall is via branches of the ventral primary rami of the lower 6 thoracic spinal nerves (includes the subcostal nerve), plus the iliohypogastric and ilioinguinal branches of the ventral primary rami of L1.

The major **arterial blood supply** to the anterior wall is derived from the superior epigastric branch of the internal thoracic artery, as well as the inferior epigastric and the deep circumflex iliac branches of the external iliac artery.

Venous drainage from the anterior wall is to the superficial epigastric, the lateral thoracic veins superiorly and the great saphenous vein inferiorly.

Lymph drainage from tissues of the anterior wall is to axillary nodes superiorly and to superficial inguinal nodes inferiorly.

INGUINAL REGION AND CANAL

The **inguinal canal** is the oblique passageway (approximately 4 cm long) in the lower aspect of the anterior abdominal wall running parallel and superior to the medial half of the inguinal ligament. Clinically, the inguinal region is important because it is the area where inguinal hernias occur.

The entrance into the canal is the **deep inguinal ring**, located just lateral to the inferior epigastric vessels and immediately superior to the midpoint of the inguinal ligament.

The **superficial inguinal ring** is the medial opening of the canal superolateral to the pubic tubercle.

Contents of the Inguinal Canal

Female Inguinal Canal

Round ligament of the uterus extends between the uterus and labia majora, and is a remnant of the caudal genital ligament and the homologue of the gubernaculum testis of the male.

Ilioinguinal nerve (L1), a branch of the lumbar plexus, exits the superficial ring to supply the skin of the anterior part of the mons pubis and labia majora.

Male Inguinal Canal

Ilioinguinal nerve (L1), a branch of the lumbar plexus, exits the superficial ring to supply the skin of the lateral and anterior scrotum.

The spermatic cord is formed during descent of the testis and contains structures that are related to the testis. The cord begins at the deep ring and courses through the inguinal canal and exits the superficial ring to enter the scrotum. The cord contains the following:

- **Testicular artery**, branch of the abdominal aorta that supplies the testis

- **Pampiniform venous plexus**, an extensive network of veins draining the testis located within the scrotum and spermatic cord. The veins of the plexus coalesce to form the testicular vein at the deep ring. The venous plexus assists in the regulation of the temperature of the testis.
- **Vas deferens** (ductus deferens) and its artery
- **Autonomic nerves**
- **Lymphatics:** Lymphatic drainage of the testis will drain into the lumbar (aortic) nodes of the lumbar region and not to the superficial inguinal nodes which drain the rest of the male perineum.

There are **3 fascial components derived from the layers** of the abdominal wall that surround the spermatic cord:

- **External spermatic fascia** is formed by the aponeuroses of the **external abdominal oblique** muscle at the superficial ring.
- **Middle or cremasteric muscle and fascia** are formed by fibers of the **internal abdominal oblique** within the inguinal canal. The cremasteric muscle elevates the testis and helps regulate the thermal environment of the testis.
- **Internal spermatic fascia** is formed by the **transversalis fascia** at the deep ring.

Boundaries of the Inguinal Canal

The **roof** is formed by fibers of the internal abdominal oblique and the transversus abdominis muscles arching over the spermatic cord.

The **anterior wall** is formed by aponeurosis of the external abdominal oblique throughout the inguinal canal and the internal abdominal oblique muscle laterally.

The **floor** formed by **inguinal ligament** throughout the entire inguinal canal and the lacunar ligament at the medial end.

The **posterior wall** is divided into lateral and medial areas:

- **Lateral area** is formed by the **transversalis fascia** and represents the **weak area** of the posterior wall.
- **Medial area** is formed and reinforced by the fused aponeurotic fibers of the internal abdominal oblique and transversus abdominis muscles (**conjoint tendon**).
- **Inferior epigastric artery and vein** ascend the posterior wall just lateral to the weak area and just medial to the deep ring.

Descent of the Testes

The testis develops from the mesoderm of the urogenital ridge within the extraperitoneal connective tissue layer. During the last trimester, it descends the posterior abdominal wall inferiorly toward the deep inguinal ring guided by the fibrous **gubernaculum**.

Clinical Correlate

A **varicocele** develops when blood collects in the pampiniform venous plexus and causes dilated and tortuous veins. This may result in swelling and enlargement of the scrotum or enlargement of the spermatic cord above the scrotum. Varicoceles are more prominent when standing because of the blood pooling into the scrotum. A varicocele will reduce in size when the individual is horizontal.

Clinical Correlate

Cancers of the **penis** and **scrotum** will metastasize to the **superficial inguinal lymph nodes,** and **testicular cancer** will metastasize to the **aortic (lumbar) nodes**.

Clinical Correlate

In males, a **cremasteric reflex** can be demonstrated by lightly touching the skin of the upper medial thigh, resulting in a slight elevation of the testis. The sensory fibers of the reflex are carried by the L1 fibers of the **ilioinguinal nerve** and the motor response is a function of the genital branch of the **genitofemoral nerve** that innervates the cremasteric muscle.

Clinical Correlate

Failure of one or both of the testes to descend completely into the scrotum results in **cryptorchidism,** which may lead to sterility if bilateral.

- An evagination of the parietal peritoneum and the peritoneal cavity extends into the inguinal canal called the **processus vaginalis**. The open connection of the processus vaginalis with the peritoneal cavity closes before birth.
- A portion of the processus vaginalis remains patent in the scrotum and surrounds the testis as the **tunica vaginalis.**

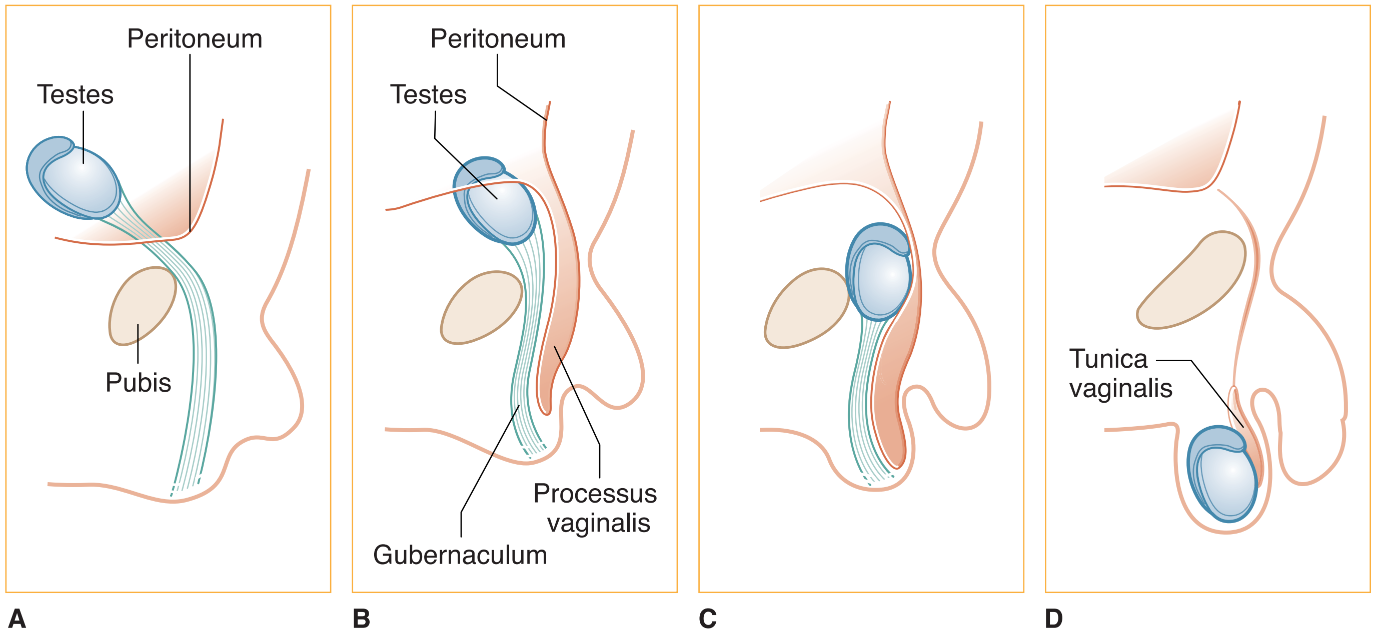

Figure II-3-4. Descent of the Testes

Clinical Correlate

A persistent process vaginalis often results in a **congenital indirect inguinal hernia.**

Clinical Correlate

A collection of serous fluid in the tunica vaginalis forms a **hydrocele,** resulting in an enlarged scrotum. A hydrocele does not reduce in size when the patient is lying down.

Note

Direct hernias are found **medial** to the inferior epigastric vessels, and indirect inguinal hernias occur **lateral** to the inferior epigastric vessels.

Inguinal Hernias

Herniation of abdominal viscera can occur in one of several weak aspects of the abdominal wall (e.g. inguinal, femoral, umbilical, or diaphragmatic). Inguinal hernias are the most common of the abdominal hernias and occur more frequently in males due to the inherent weakness of the male inguinal canal. Inguinal hernias occur superior to the inguinal ligament.

- **Indirect inguinal hernias** result when abdominal contents protrude through the deep inguinal ring **lateral** to the inferior epigastric vessels. After passing through the inguinal canal and superficial ring, the viscera can continue and coil in the scrotum.
  - Indirect hernias follow the route taken by the testis and are found **within** the spermatic cord.
  - They are covered by the 3 layers of spermatic fascia.
- **Direct inguinal hernias** result when the abdominal contents protrude through the weak area of the posterior wall of the inguinal canal **medial** to the inferior epigastric vessels (in the **inguinal [Hesselbach's] triangle**).

– Direct hernias rupture through the posterior wall of the inguinal canal and are usually found on the surface of the spermatic cord and bulge at the superficial ring.

– They may be covered by only the external layer of spermatic fascia.

Clinical Correlate

Direct inguinal hernias usually pass through the **inguinal (Hesselbach's) triangle:**

- **Lateral border:** inferior epigastric vessels
- **Medial border:** rectus abdominis muscle
- **Inferior border:** inguinal ligament

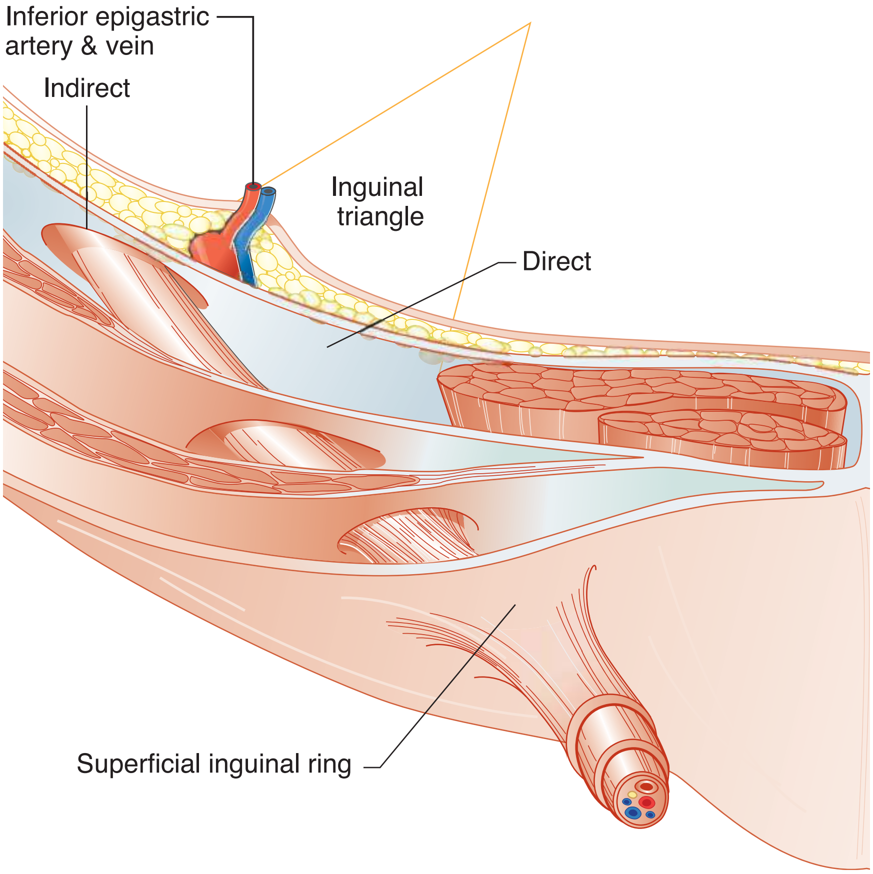

Figure II-3-5. Inguinal Hernia

Note

Both direct and indirect hernias exit through the superficial ring, but only indirect hernias pass through the deep ring.

Clinical Correlate

- Inguinal hernias pass **above** the inguinal ligament.
- Femoral hernias pass **below** the inguinal ligament.

Femoral Hernias

Femoral hernias most often occur in women.

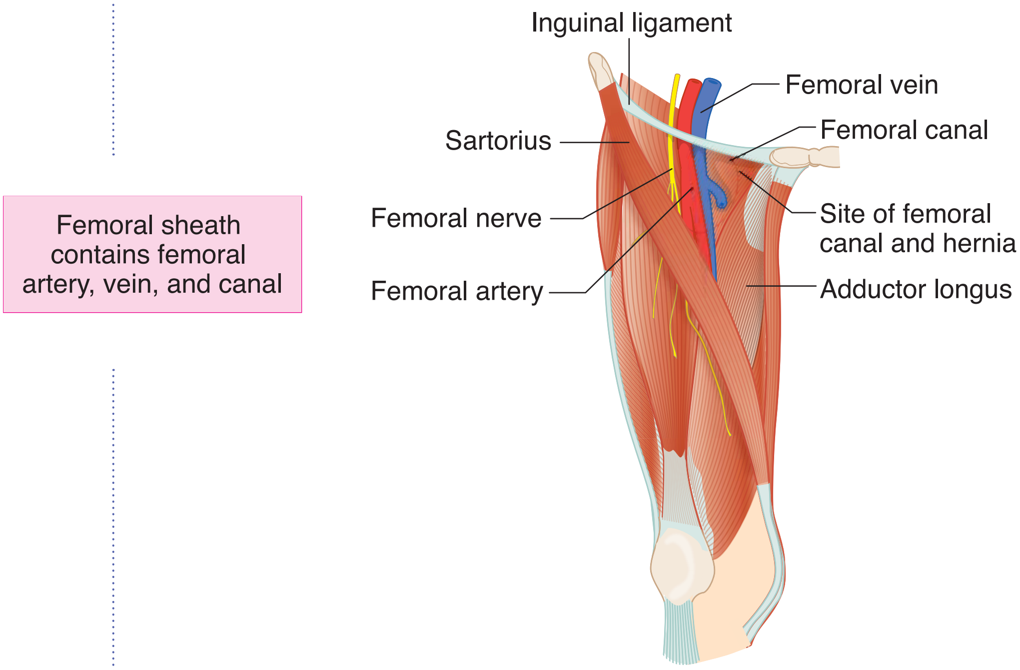

Figure II-3-6. Femoral Hernia

EMBRYOLOGY OF THE GASTROINTESTINAL SYSTEM

Primitive Gut Tube

The primitive gut tube is formed by incorporation of the **yolk sac** into the embryo during 2 body foldings: **head to tail (cranial-caudal)** and **lateral foldings**. While the epithelial lining of the mucosa of the primitive gut tube is derived from **endoderm**, the lamina propria, muscularis mucosae, submucosa, muscularis externa, and adventitia/serosa are derived from **mesoderm**.

The primitive gut tube is divided into **the foregut, midgut, and hindgut**, each supplied by a specific artery and autonomic nerves.

Table II-3-1. Adult Structures Derived from the 3 Divisions of the Primitive Gut Tube

Foregut	Midgut	Hindgut
Artery: celiac **Parasympathetic innervation:** vagus nerves **Sympathetic innervation:** • Preganglionics: thoracic splanchnic nerves, T5–T9 • Postganglionic cell bodies: celiac ganglion	**Artery:** superior mesenteric **Parasympathetic innervation:** vagus nerves **Sympathetic innervation:** • Preganglionics: thoracic splanchnic nerves, T9–T12 • Postganglionic cell bodies: superior mesenteric ganglion	**Artery:** inferior mesenteric **Parasympathetic innervation:** pelvic splanchnic nerves **Sympathetic innervation:** • Preganglionics: lumbar splanchnic nerves, L1–L2 • Postganglionic cell bodies: inferior mesenteric ganglion
Referred Pain: Epigastrium	**Referred Pain:** Umbilical	**Referred Pain:** Hypogastrium
Foregut Derivatives	**Midgut Derivatives**	**Hindgut Derivatives**
Esophagus Stomach Duodenum (first and second parts) Liver Pancreas Biliary apparatus Gallbladder	Duodenum (second, third, and fourth parts) Jejunum Ileum Cecum Appendix Ascending colon Transverse colon (proximal two-thirds)	Transverse colon (distal third—splenic flexure) Descending colon Sigmoid colon Rectum Anal canal (above pectinate line)

Development and Rotation of Foregut

After body foldings and the formation of the gut tube, the foregut is suspended from the dorsal body wall by the **dorsal embryonic mesentery** and from the ventral body wall by the **ventral embryonic mesentery**. Note that the **liver** develops in the ventral embryonic mesentery, and the **spleen** and dorsal pancreatic bud develop in the dorsal embryonic mesentery.

- The abdominal foregut rotates 90° (clockwise) around its longitudinal axis. The original left side of the stomach before rotation becomes the ventral surface after rotation and its anterior and posterior borders before rotation will become the **lesser and greater curvatures**, respectively.
- Foregut rotation results in the liver, lesser omentum (ventral embryonic mesentery), pylorus of the stomach, and duodenum moving to the **right**; and the spleen, pancreas, and greater omentum (dorsal embryonic mesentery) moving to the **left**.
- The ventral embryonic mesentery will contribute to the **lesser omentum** and **the falciform ligament**, both of which attach to the liver.
- The dorsal embryonic mesentery will contribute to the **greater omentum** and the **gastro-splenic** and **splenorenal** ligaments, all of which attach to the spleen or the greater curvature of the stomach.

Note

The lower respiratory tract, liver and biliary system, and pancreas all develop from an **endodermal outgrowth of the foregut**.

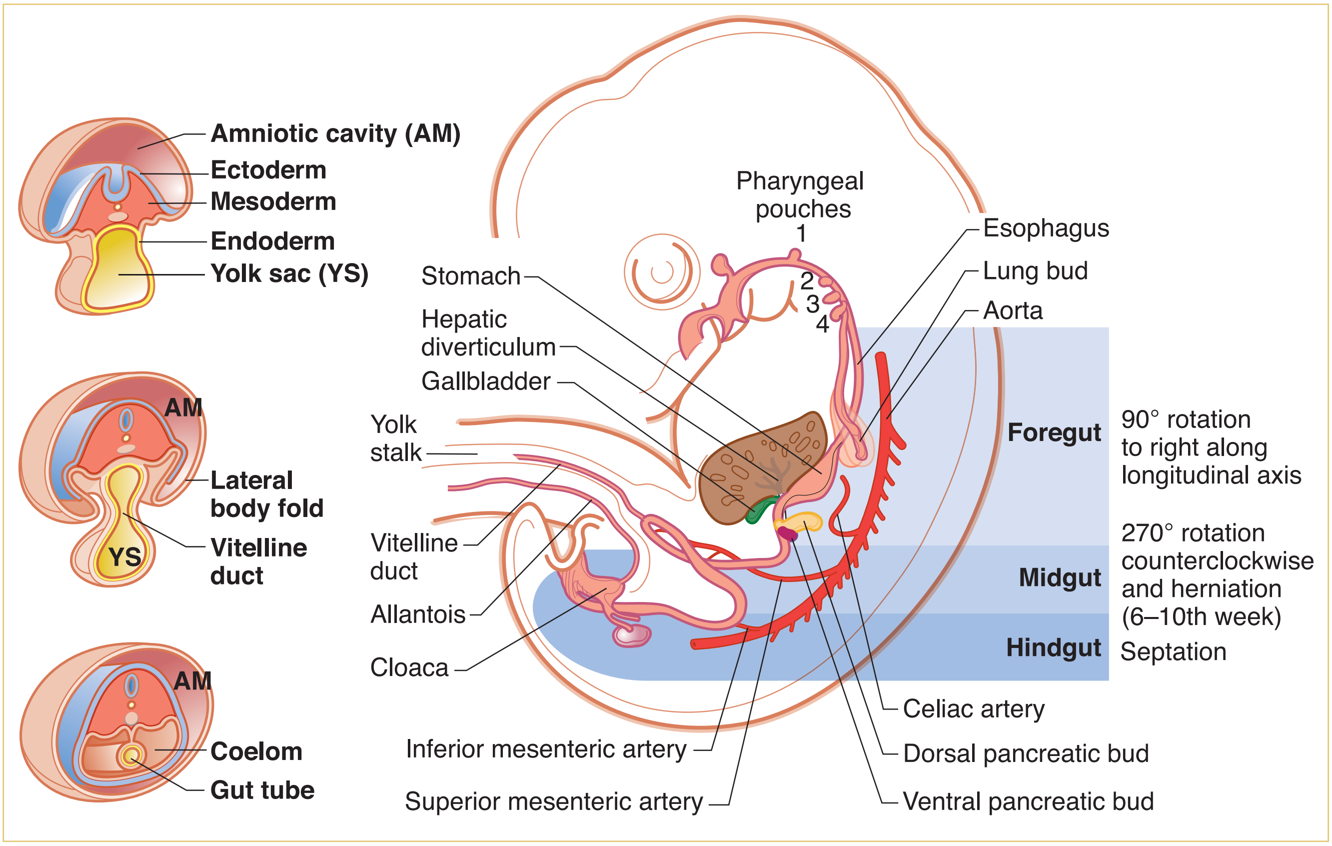

Figure II-3-7A. Development of Gastrointestinal Tract

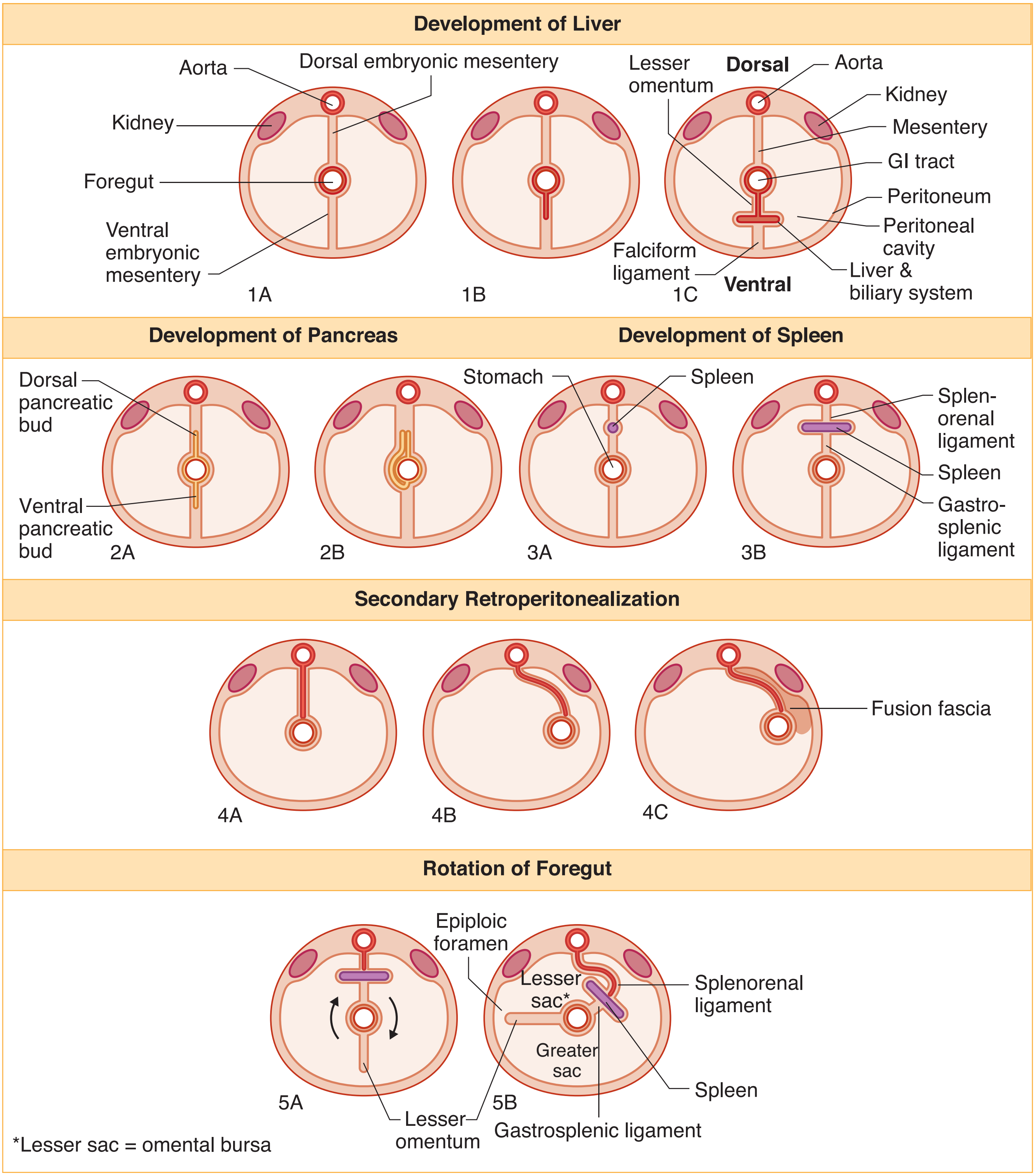

Figure II-3-7B. Cross-Sectional View of Foregut Development and Rotation

Development and Rotation of Midgut

The midgut originally develops from the cranial and caudal intestinal loops. During weeks 6–10, the midgut herniates into the umbilical cord. During herniation into and return from the umbilical cord, the midgut undergoes a 270° counterclockwise rotation around the axis of the **superior mesenteric artery**.

- This rotation results in the jejunum being on the left, and the ileum and cecum being on the right.
- It also causes the colon to assume the shape of an inverted "U."

PERITONEUM

The peritoneum is the serous membrane related to the viscera of the abdominal cavity. It is divided into 2 layers: **parietal** and **visceral**.

Clinical Correlate

Inflammation of the parietal peritoneum (peritonitis) results in sharp pain that is localized over the area.

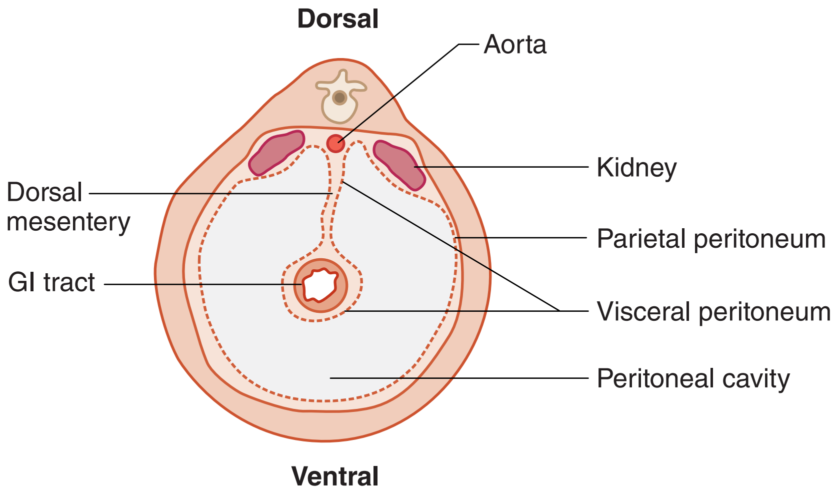

Figure II-3-7C. Peritoneum

The **parietal layer** lines the body wall and covers the retroperitoneal organs on one surface. Parietal peritoneum is very sensitive to somatic pain and is innervated by the lower intercostal nerves and the ilioinguinal and the iliohypogastric nerves of the lumbar plexus.

The **visceral layer** encloses the surfaces of the intraperitoneal organs. The visceral peritoneum usually forms double-layered peritoneal membranes (mesenteries) that suspend parts of the GI tract from the body wall. The mesenteries allow for the passage of vessels, nerves, and lymphatics to reach the GI tract.

Different terms describe the postnatal remnants of mesenteries in the abdomen (Figures II-3-8 and II-3-9):

- **Omentum**: lesser and greater omenta attach to the lesser or greater curvatures of the stomach, respectively
- **Mesocolon**: transverse and sigmoid mesocolon attach to the transverse colon or the sigmoid colon, respectively
- **Ligaments**: reflections of mesenteries between organs or the body wall, named according to their attachments

Peritoneal Cavity

The peritoneal cavity is the potential space located between the parietal and visceral peritoneal layers. The 90° rotation and the shift of the embryonic mesenteries divide the peritoneal cavity into 2 sacs (Figures II-3-7B and II-3-9).

- **The lesser sac (omental bursa)** is a cul-de-sac formed posterior to the stomach and the lesser omentum
- **The greater sac** is formed by the larger area of the remaining peritoneal cavity. The only communication between the lesser sac and the greater sac is the **epiploic foramen (of Winslow)**.

Intraperitoneal versus Retroperitoneal Organs

The abdominal viscera are classified according to their relationship to the peritoneum.

- **Intraperitoneal organs** are suspended by a mesentery and are almost completely enclosed in visceral peritoneum. They are mobile.
- **Retroperitoneal organs** are partially covered on one side with parietal peritoneum. They are immobile or fixed. Many retroperitoneal organs are originally suspended by a mesentery and become secondarily retroperitoneal. In **secondary retroperitonealization,** parts of the gut tube (most of the duodenum, pancreas, ascending colon, descending colon, part of rectum) fuse with the body wall by way of fusion of visceral peritoneum with parietal peritoneum. This causes the organs to become secondarily retroperitoneal (and the visceral peritoneum covering the organ is renamed as the parietal peritoneum). The vessels within the mesentery of these gut structures become secondarily retroperitoneal.

Table II-3-2. Intraperitoneal and Retroperitoneal Organs

Major Intraperitoneal Organs (suspended by a mesentery)	**Major Secondary Retroperitoneal Organs** (lost a mesentery during development)	**Major Primary Retroperitoneal Organs** (never had a mesentery)
Stomach	Duodenum, 2nd and 3rd parts	Kidneys
Liver and gallbladder	Head, neck, and body of pancreas	Adrenal glands
Spleen	Ascending colon	Ureters
Duodenum, 1st part	Descending colon	Aorta
Tail of pancreas	Upper rectum	Inferior vena cava
Jejunum		Lower rectum
Ileum		Anal canal
Appendix		
Transverse colon		
Sigmoid colon		

Epiploic Foramen (of Winslow)

The **epiploic foramen** is the opening between omental bursa and greater peritoneal sac (Figures II-3-7B, II-3-8, and II-3-9). The boundaries of the epiploic foramen are the following:

- **Anteriorly:** hepatoduodenal ligament and the hepatic portal vein
- **Posteriorly:** inferior vena cava
- **Superiorly:** caudate lobe of the liver
- **Inferiorly:** first part of the duodenum

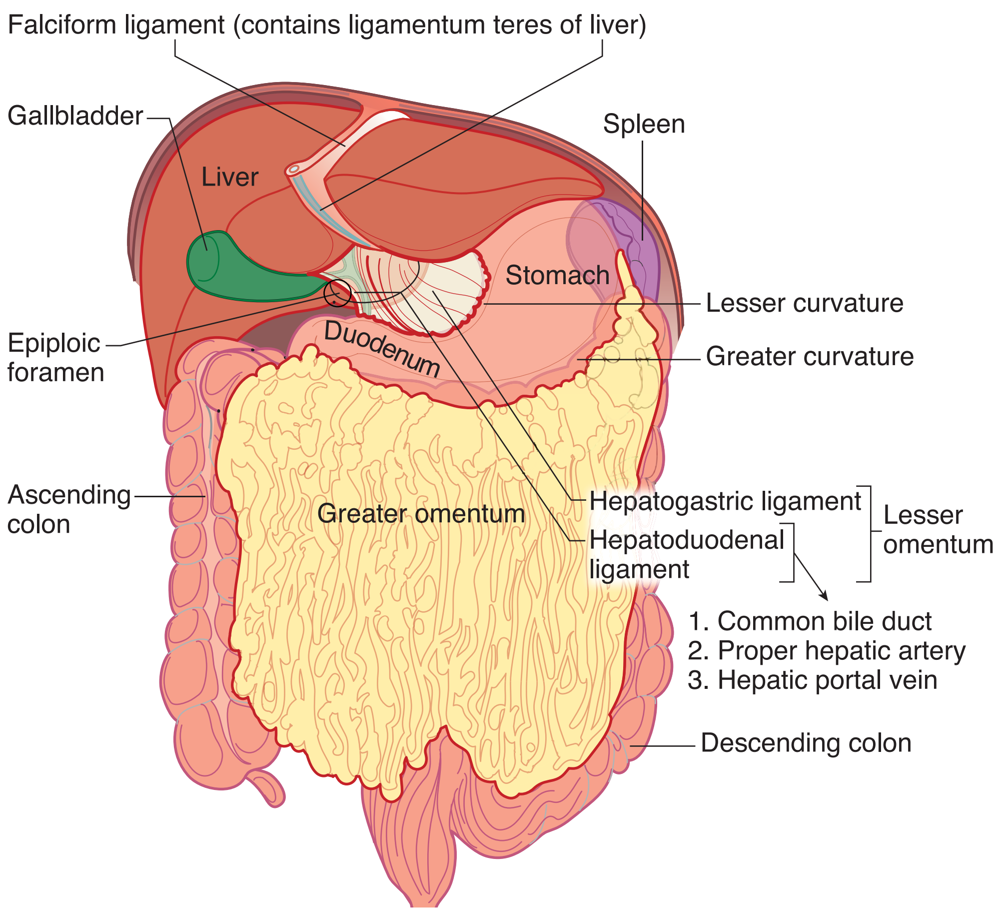

Figure II-3-8. Peritoneal Membranes

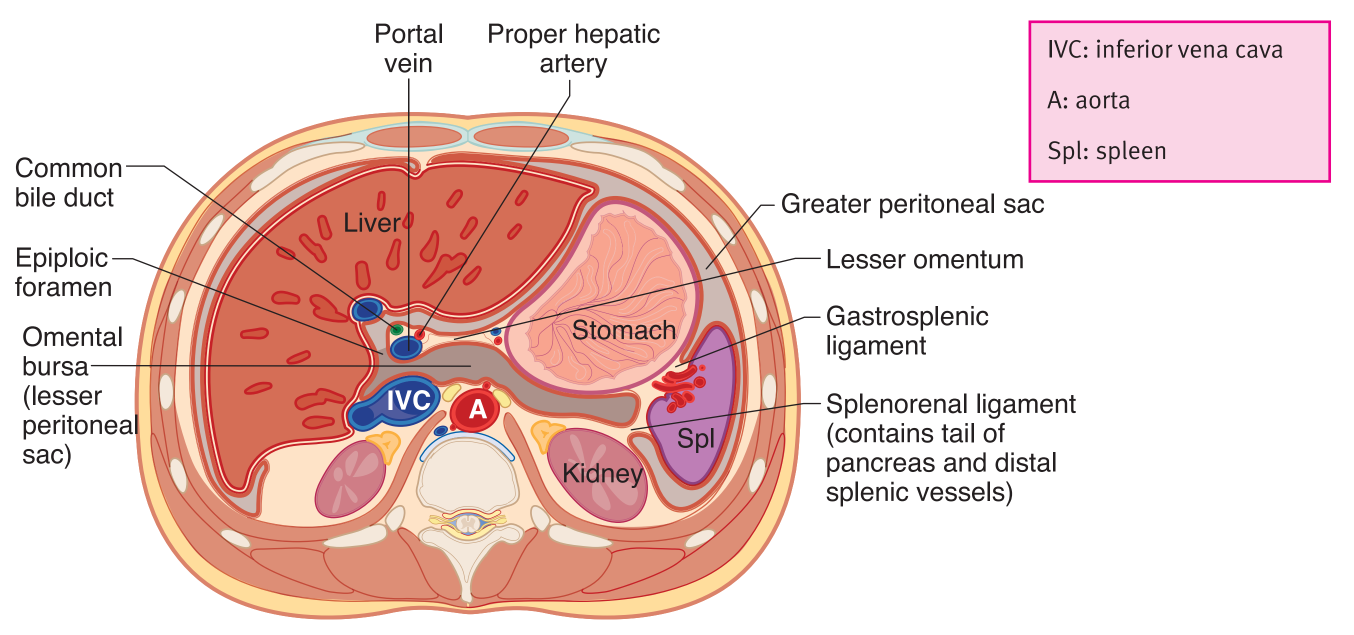

Figure II-3-9. Greater and Lesser Peritoneal Sacs

DEVELOPMENT OF ABDOMINAL VISCERA

Liver

The hepatic diverticulum develops as an outgrowth of the **endoderm** of the foregut in the region of the duodenum near the border between the foregut and the midgut.

- This diverticulum enters the ventral embryonic mesentery and distally becomes the liver and gallbladder and proximately becomes the biliary duct system.
- The part of the ventral embryonic mesentery between the liver and gut tube becomes the **lesser omentum**, and the part between the liver and ventral body wall becomes the **falciform ligament**.

Pancreas

The pancreas develops from 2 pancreatic diverticula (buds), which evaginate from the **endodermal** lining of the foregut in the region of duodenum.

- The ventral bud rotates around the gut tube to fuse with the dorsal bud, together to form a single pancreas.
- The **dorsal pancreatic bud** forms the **neck, body, and tail of the pancreas**.
- The **ventral pancreatic bud** forms the **head and uncinate process**.

Clinical Correlate

A defect in the rotation and fusion of the ventral and dorsal buds results in an **annular pancreas** which can **constrict or obstruct the duodenum** and result **in polyhydramnios**.

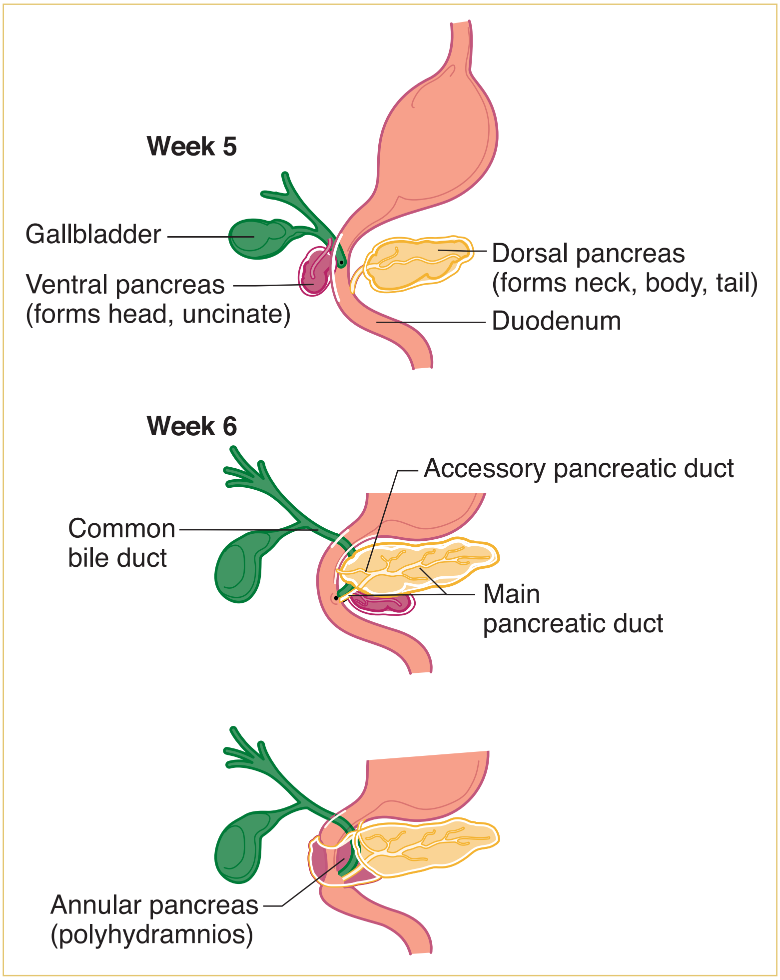

Figure II-3-10. Development of the Pancreas and Duodenum

Spleen

The spleen develops from **mesoderm** within the dorsal embryonic mesentery (Figure II-3-7B). The embryonic mesentery between the spleen and the gut tube becomes the **gastrosplenic ligament**. The mesentery between the spleen and the dorsal body wall becomes the **splenorenal ligament**.

CONGENITAL ABNORMALITIES OF THE GUT TUBE

Hypertrophic pyloric stenosis occurs when the muscularis externa hypertrophies, causing a narrow pyloric lumen. It is associated with **polyhydramnios**; projectile, nonbilious vomiting; and a small knot at the right costal margin.

Extrahepatic biliary atresia occurs when the lumen of the biliary ducts is occluded owing to incomplete recanalization. It is associated with jaundice, white-colored stool, and dark-colored urine.

Annular pancreas occurs when the ventral and dorsal pancreatic buds form a ring around the duodenum, thereby causing an obstruction of the duodenum and polyhydramnios.

Duodenal atresia occurs when the lumen of the duodenum is occluded owing to failed recanalization. It is associated with **polyhydramnios**, bile-containing vomitus, and a distended stomach.

An **omphalocele** occurs when the midgut loop fails to return to the abdominal cavity and remains in the umbilical stalk. The viscera herniate through the **umbilical ring** and are contained in a **shiny sac** of amnion at the base of the umbilical cord. It is often associated with multiple anomalies of the heart and nervous system, with a high mortality rate (25%).

Gastroschisis occurs when the abdominal viscera herniate through the body wall directly into the amniotic cavity, usually to the right of the umbilicus.

- This is a defect in the closure of the lateral body folds and a weakness of the anterior wall.
- Note that the viscera do not protrude through the umbilical ring and are not enclosed in a sac of amnion.

Ileal (Meckel) diverticulum occurs when a remnant of the vitelline duct persists, thereby forming a blind pouch on the antimesenteric border of the ileum. It is often asymptomatic but can become inflamed if it contains ectopic gastric, pancreatic, or endometrial tissue, which may produce ulceration. It is typically found 2 feet from the ileocecal junction, are 2 inches long, and appears in 2% of the population.

Vitelline fistula occurs when the vitelline duct persists, thereby forming a direct connection between the intestinal lumen and the outside of the body at the umbilicus. It is associated with drainage of meconium from the umbilicus.

Malrotation of midgut occurs when the midgut undergoes only partial rotation and results in abnormal position of abdominal viscera. It may be associated with **volvulus** (twisting of intestines).

Colonic aganglionosis (Hirschsprung disease) results from the failure of **neural crest** cells to form the myenteric plexus in the sigmoid colon and rectum. It is associated with loss of peristalsis and immobility of the hindgut, fecal retention and abdominal distention of the transverse colon (megacolon).

ABDOMINAL VISCERA

Liver

The liver has 2 surfaces: a superior or **diaphragmatic** surface and an inferior or a **visceral** surface. It lies mostly in the right aspect of the abdominal cavity and is protected by the rib cage. The liver is invested by visceral peritoneum:

- The reflection of visceral peritoneum between the diaphragmatic surface of the liver and the diaphragm forms the **falciform ligament**, which continues onto the liver as the coronary ligament and the right and left triangular ligaments.
- The extension of visceral peritoneum between the visceral surface of the liver and the first part of the duodenum and the lesser curvature of the stomach forms the **hepatoduodenal and hepatogastric ligaments** of the **lesser omentum**, respectively.

The liver is divided into 2 lobes of unequal size:

- Fissures for the ligamentum teres and the ligamentum venosum, the porta hepatis, and the fossa for the gallbladder further subdivide the right lobe into the right lobe proper, the quadrate lobe, and the caudate lobe.
- The quadrate and caudate lobes are anatomically part of the right lobe but functionally part of the left. They receive their blood supply from the left branches of the portal vein and hepatic artery and secrete bile to the left hepatic duct.

The liver has a central hilus, or porta hepatis, which receives venous blood from the **portal vein** and arterial blood from the **hepatic artery**.

- The central hilus also transmits the common bile duct, which collects bile produced by the liver.
- These structures, known collectively as the portal triad, are located in the hepatoduodenal ligament, which is the right free border of the lesser omentum.

The **hepatic veins** drain the liver by collecting blood from the liver sinusoids and returning it to the inferior vena cava.

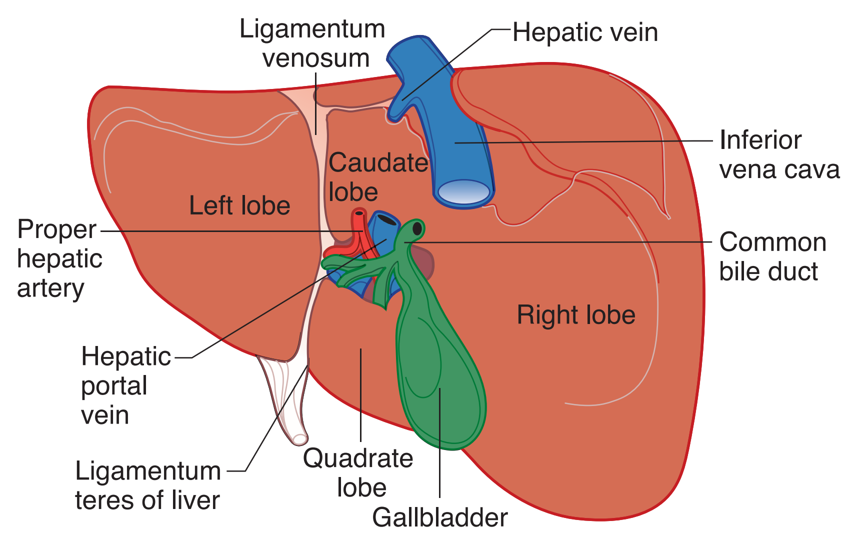

Figure II-3-11. Visceral Surface of the Liver

Gallbladder

The gallbladder lies in a fossa on the visceral surface of the liver to the right of the quadrate lobe. It stores and concentrates bile, which enters and leaves through the cystic duct.

- The cystic duct joins the common hepatic duct to form the **common bile duct**.
- The common bile duct descends in the hepatoduodenal ligament, then passes posterior to the first part of the duodenum. The common bile duct

penetrates the head of the pancreas where it joins the main pancreatic duct and forms the **hepatopancreatic ampulla,** which drains into the second part of the duodenum at the **major duodenal papilla**.

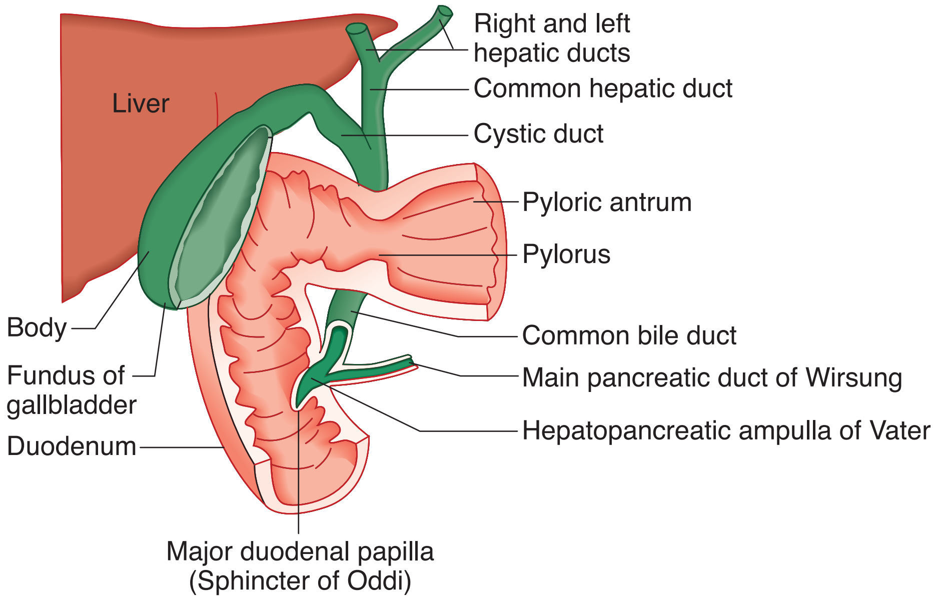

Figure II-3-12. Biliary Ducts

Pancreas

The pancreas horizontally crosses the posterior abdominal wall at approximately at the level of the transpyloric plane. The gland consists of 4 parts: **head, neck, body, and tail**.

- The head of the pancreas rests within the C-shaped area formed by the duodenum and is traversed by the common bile duct. It includes the **uncinate process** which is crossed by the superior mesenteric vessels.
- Posterior to the neck is the site of formation of the hepatic portal vein.
- The body passes to the left and passes anterior to the aorta and the left kidney. The splenic artery undulates along the superior border of the body of the pancreas with the splenic vein coursing posterior to the body.
- The tail of the pancreas enters the **splenorenal ligament** to reach the hilum of the spleen. The tail is the only part of the pancreas that is **intraperitoneal**.

The main **pancreatic duct** (Figures II-3-12 and II-3-13) courses through the body and tail of the pancreas to reach the head of the pancreas, where it joins with the common bile duct to form the hepatopancreatic ampulla.

The head of the pancreas receives its blood supply from the **superior and inferior pancreaticoduodenal branches** of the gastroduodenal and superior mesenteric arteries, respectively. This region is important for **collateral circulation** because there are anastomoses between these branches of the celiac trunk and superior mesenteric artery.

Clinical Correlate

Carcinoma of the pancreas commonly occurs in the **head of the pancreas** and may constrict the main **pancreatic duct** and the **common bile duct**. Obstruction of the bile duct may cause **jaundice**.

The neck, body, and tail of the pancreas receive their blood supply from the **splenic artery**.

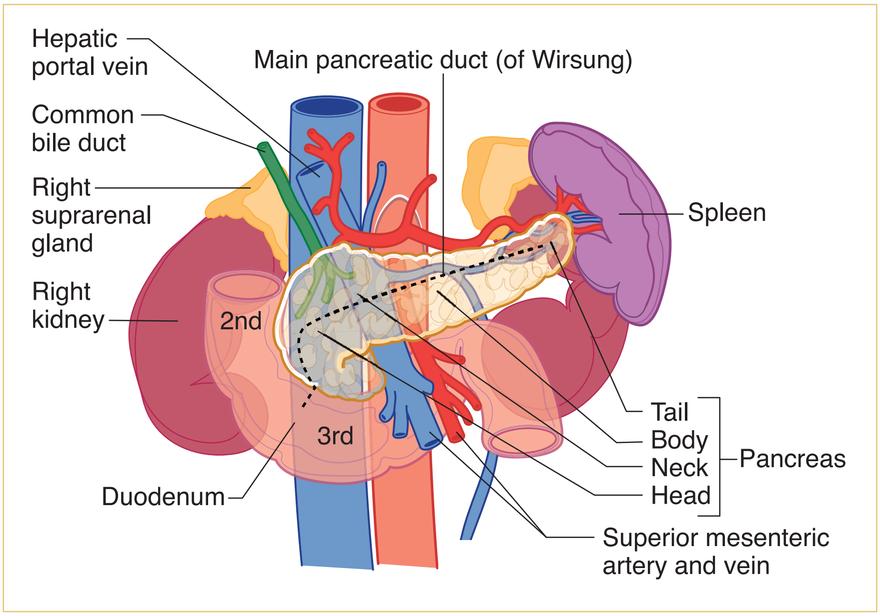

Figure II-3-13. Adult Pancreas

Clinical Correlate

The spleen may be lacerated with a fracture of the **9th, 10th, or 11th rib on the left side.**

Spleen

The spleen is a peritoneal organ in the upper left quadrant that is deep to the left 9th, 10th, and 11th ribs. The visceral surface of the spleen is in contact with the left colic flexure, stomach, and left kidney. Inasmuch as the spleen lies above the costal margin, a normal-sized spleen is not palpable.

The splenic artery and vein reach the hilus of the spleen by traversing the **splenorenal ligament**.

Clinical Correlate

A **sliding hiatal hernia** occurs when the cardia of the stomach herniates through the esophageal hiatus of the diaphragm. This can damage the vagal trunks as they pass through the hiatus.

Stomach

The stomach has a right **lesser curvature**, which is connected to the porta hepatis of the liver by the lesser omentum (hepatogastric ligament), and a left **greater curvature** from which the greater omentum is suspended (Figure II-3-8).

The cardiac region receives the esophagus; and the dome-shaped upper portion of the stomach, which is normally filled with air, is the fundus. The main central part of the stomach is the body. The pyloric portion of the stomach has a thick muscular wall and narrow lumen that empties into the duodenum approximately in the transpyloric plane (L1 vertebra).

Duodenum

The duodenum is C-shaped, has 4 parts, and is located retroperitoneal except for the first part.

- The first part is referred to as the duodenal cap (bulb). The gastroduodenal artery and the common bile duct descend posterior to the first part.
- The second part (descending) receives the **common bile duct** and main **pancreatic duct** at the hepatopancreatic ampulla (of Vater). Smooth muscle in the wall of the duodenal papilla is known as the sphincter of Oddi.

Note that the foregut terminates at the point of entry of the common bile duct; the remainder of the duodenum is part of the midgut.

Jejunum and Ileum

The jejunum begins at the duodenojejunal junction and comprises 2/5 of the remaining small intestine. The beginning of the ileum is not clearly demarcated; it consists of the distal 3/5 of the small bowel.

The jejunoileum is suspended from the posterior body wall by the mesentery proper. Although the root of the mesentery is only 6 inches long, the mobile part of the small intestine is approximately 22 feet in length.

Colon

The **cecum** is the first part of the colon, or large intestine, and begins at the ileocecal junction. It is a blind pouch, which often has a mesentery and gives rise to the vermiform appendix. The appendix has its own mesentery, the mesoappendix.

The **ascending colon** lies retroperitoneally and lacks a mesentery. It is continuous with the transverse colon at the right (hepatic) flexure of colon.

The **transverse colon** has its own mesentery called the **transverse mesocolon**. It becomes continuous with the descending colon at the left (splenic) flexure of colon. The midgut terminates at the junction of the proximal two-thirds and distal one-third of the transverse colon.

The **descending colon** lacks a mesentery. It joins the sigmoid colon where the large bowel crosses the pelvic brim.

The **sigmoid colon** is suspended by the **sigmoid mesocolon**. It is the terminal portion of the large intestine and enters the pelvis to continue as the rectum.

The superior one-third of the **rectum** is covered by peritoneum anteriorly and laterally. It is the fixed, terminal, straight portion of the hindgut.

The **anal canal** is about 1.5 inches long and opens distally at the anus. It is continuous with the rectum at the pelvic diaphragm, where it makes a 90-degree posterior bend (anorectal flexure) below the rectum.

- The **puborectalis component** of the pelvic diaphragm pulls the flexure forward, helping to maintain fecal continence.
- The **internal anal sphincter** is circular smooth muscle that surrounds the anal canal. The **sympathetics** (lumbar splanchnics) **increase** the tone of the muscle and the **parasympathetics** (pelvic splanchnics) **relax** the muscle during defecation.

- The **external anal sphincter** is circular voluntary skeletal muscle surrounding the canal that is voluntarily controlled by the inferior rectal branch of the **pudendal nerve** and **relaxes** during defecation.
- The anal canal is divided into upper and lower parts separated by the **pectinate line**, an elevation of the mucous membrane at the distal ends of the anal columns.

Table II-3-3. Comparison of Features Above and Below the Pectinate Line

Above	Below
Visceral (ANS) sensory innervation	Somatic sensory innervation
Portal venous drainage	Caval venous drainage
Drain to iliac lymph nodes	Drain to superficial inguinal nodes
Internal hemorrhoids (painless)	External hemorrhoids (painful)
Endoderm	Ectoderm

Abbreviations: ANS, autonomic nervous system

GASTROINTESTINAL HISTOLOGY

The alimentary or gastrointestinal (GI) tract is a muscular tube that runs from the oral cavity to the anal canal. The GI tract walls are composed of 4 layers: mucosa, submucosa, muscularis externa, and serosa.

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Figure II-3-14. Organization of the GI tract

Mucosa (M) submucosa (SB), muscularis externa (ME), serosa or visceral peritoneum (S), mesentery (arrow)

Mucosa

The **mucosa** is the innermost layer and has 3 components.

- The **epithelium** lining the lumen varies in different regions, depending on whether the function is primarily conductive and protective (stratified squamous; in the pharynx and esophagus), or secretory and absorptive (simple columnar; stomach and intestine).
- The **lamina propria** is a layer of areolar connective tissue that supports the epithelium and attaches it to the underlying muscularis mucosae. Numerous capillaries form extensive networks in the lamina propria (particularly in the small intestine).

  Within the lamina propria are blind-ended lymphatic vessels (lacteals) that carry out absorbed nutrients and white blood cells (particularly lymphocytes). The GALT (gut-associated lymphoid tissue), responsible for IgA production, is located within the lamina propria.
- The **muscularis mucosa** is a thin smooth-muscle layer that marks the innermost edge of the mucosa. The muscle confers some motility to the mucosa and facilitates discharge of secretions from glands. In the small intestine, a few strands of smooth muscle may run into the lamina propria and up to the tips of villi.

Submucosa

The **submucosa** is a layer of loose areolar connective tissue that attaches the mucosa to the muscularis externa and houses the larger blood vessels and mucous-secreting glands.

Muscularis Externa

The **muscularis externa** is usually comprised of 2 layers of muscle: an inner circular and an outer longitudinal. The muscularis externa controls the lumen size and is responsible for peristalsis. The muscle is striated in the upper third of the esophagus and smooth elsewhere.

Serosa

The **serosa** (or peritoneum of anatomy) is composed of a mesothelium (a thin epithelium lining the thoracic and abdominal cavities) and loose connective tissue. In the abdominal cavity, the serosa surrounds each intestinal loop and then doubles to form the mesentery within which run blood and lymphatic vessels.

INNERVATION

The GI tract has both intrinsic and extrinsic innervation. The **intrinsic** innervation is entirely located within the walls of the GI tract. The intrinsic system is capable of autonomous generation of peristalsis and glandular secretions. An interconnected network of ganglia and nerves located in the submucosa forms the Meissner's plexus and controls much of the intrinsic motility of the lining of the alimentary tract. Auerbach's plexus contains a second network of neuronal ganglia, and is located between the 2 muscle layers of the muscularis externa. All GI-tract smooth muscle is interconnected by gap junctions.

Clinical Correlate

Hirschsprung disease or aganglionic megacolon is a genetic disease present in approximately 1 out of 5,000 live births. It may result from mutations that affect the migration of neural crest cells into the gut. This results in a deficiency of terminal ganglion cells in Auerbach's plexus and affects digestive tract motility, particularly in the rectum (peristalsis is not as effective and constipation results).

The **extrinsic** autonomic innervation to the GI tract is from the parasympathetic (stimulatory) and sympathetic (inhibitory) axons that modulate the activity of the intrinsic innervation. Sensory fibers accompany the parasympathetic nerves and mediate visceral reflexes and sensations, such as hunger and rectal fullness. Visceral pain fibers course back to the CNS with the sympathetic innervation. Pain results from excessive contraction and/or distention of the smooth muscle. Visceral pain is referred to the body wall dermatomes that match the sympathetic innervation to that GI tract structure.

IMMUNE FUNCTIONS

The lumen of the GI tract is normally colonized by abundant bacterial flora. The majority of the bacteria in the body—comprising about 500 different species—are in our gut, where they enjoy a rich growth medium within a long, warm tube. Most of these bacteria are beneficial (vitamins B12 and K production, additional digestion, protection against pathogenic bacteria) but a few species of pathogenic microbes appear at times. The gut has defense mechanisms to fight these pathogens (GALT and Paneth cells).

REGIONAL DIFFERENCES

Major differences lie in the general organization of the mucosa (glands, folds, villi, etc.) and in the types of cells comprising the epithelia and associated glands in the GI tract.

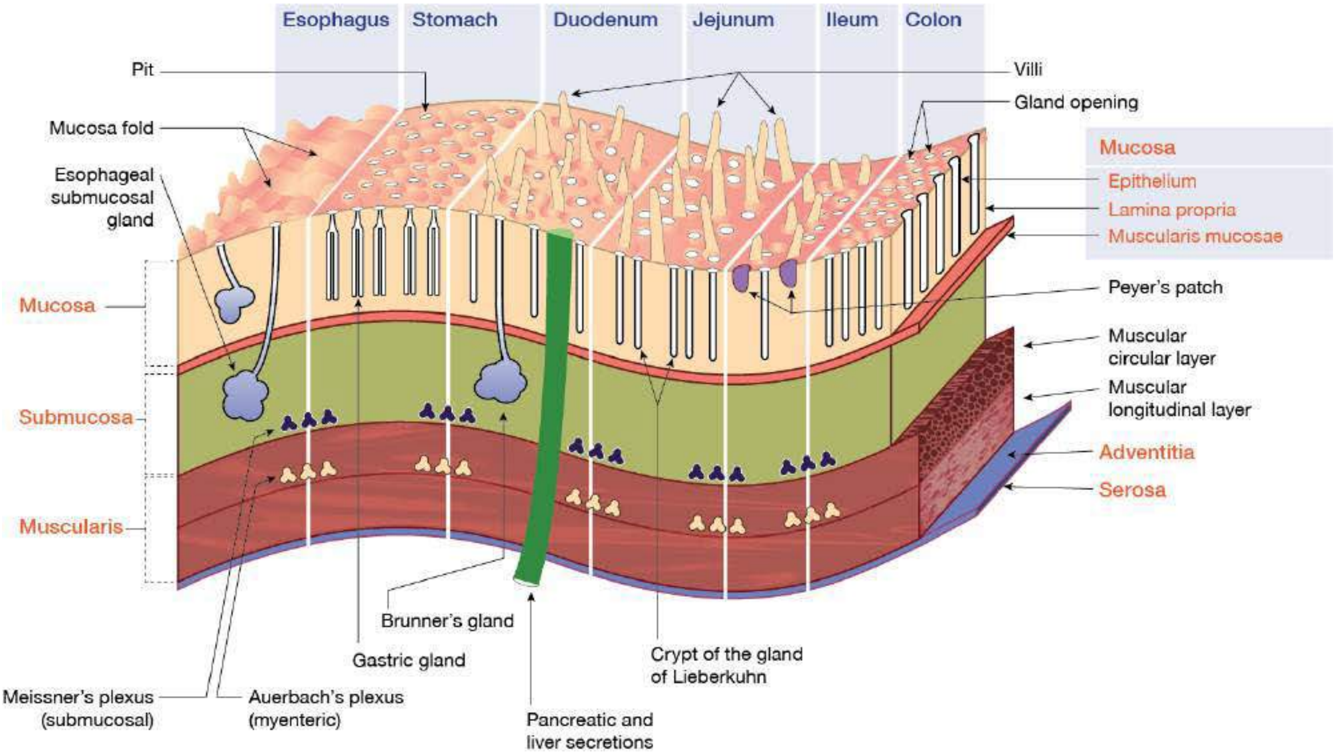

Figure II-3-15. Histologic Organization of the Digestive Tube

Table II-3-4. Histology of Specific Regions

Region	Major Characteristics	Mucosal Cell Types at Surface	Function of Surface Mucosal Cells
Esophagus	• Nonkeratinized stratified squamous epithelium • Skeletal muscle in muscularis externa (upper 1/3) • Smooth muscle (lower 1/3)	—	—
Stomach (body and fundus)	*Rugae:* shallow pits; deep glands	Mucous cells	Secrete mucous; form protective layer against acid; tight junctions between these cells probably contribute to the acid barrier of the epithelium.
		Chief cells	Secrete pepsinogen and lipase precursor
		Parietal cells	Secrete HCl and intrinsic factor
		Enteroendocrine (EE) cells	Secrete a variety of peptide hormones
Pylorus	Deep pits; shallow, branched glands	Mucous cells	Same as above
		Parietal cells	Same as above
		EE cells	High concentration of gastrin
Small intestine	Villi, plicae, and crypts	Columnar absorptive cells	Contain numerous microvilli that greatly increase the luminal surface area, facilitating absorption
Duodenum	Brunner glands, which discharge alkaline secretion	Goblet cells	Secrete acid glycoproteins that protect mucosal linings
		Paneth cells	Contains granules that contain lysozyme. May play a role in regulating intestinal flora
		EE cells	High concentration of cells that secrete cholecystokinin and secretin
Jejunum	Villi, well developed plica, crypts	Same cell types as found in the duodenal epithelium	Same as above
Ileum	Aggregations of lymph nodules called Peyer's patches	M cells found over lymphatic nodules and Peyer's patches	Endocytose and transport antigen from the lumen to lymphoid cells
Large intestine	Lacks villi, crypts	Mainly mucous-secreting and absorptive cells	Transports Na^+ (actively) and water (passively) out of lumen

Oral Cavity

The epithelium of the oral cavity is a stratified squamous epithelium. Mucous and serous secretions of the salivary glands lubricate food, rinse the oral cavity, moisten the food for swallowing and provide partial antibacterial protection. Secretions of IgA from plasma cells within the connective tissue are transported through the gland epithelia to help protect against microbial attachment and invasion.

Esophagus

The esophagus is also lined by a stratified squamous epithelium. In the lower part of the esophagus there is an abrupt transition to the simple columnar epithelium of the stomach. Langerhans cells—macrophage-like antigen-presenting cells—are present in the epithelial lining. The muscularis externa of the esophagus consists of striated muscle in the upper third, smooth muscle in the distal third, and a combination of both in the middle third.

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Figure II-3-16. Esophagus with non-keratinizing stratified squamous epithelium (arrow) and a thin lamina propria with vessels (arrowheads)

The underlying muscularis externa (ME) is skeletal muscle from the upper half of the esophagus

Stomach

The stomach has 3 distinct histological areas: the cardia, body, and pyloric antrum. The mucosa of the stomach is thrown into folds (rugae) when empty, but disappears when the stomach is full. The surface is lined by a simple columnar epithelium. The stomach begins digestion by initiating the chemical and enzymatic breakdown of ingested food. Proteins are initially denatured by the acidic gastric juice before being hydrolyzed to polypeptide fragments by the enzyme pepsin. The chyme consists of denatured and partially broken-up food particles suspended in a semi-fluid, highly acidic medium.

Gastric pits form numerous deep tubular invaginations that line the inner surface of the stomach. The gastric pits are closely spaced; they penetrate into the thickness of the mucosa and extend into gastric glands. The transition between pits and glands is marked by the isthmus, where there is a narrowing of the lumen, and by a change in cellular composition of the epithelium. The glands are coiled in the cardiac and pyloric regions of the stomach and straight in the fundus and body regions. Glands in the body of the stomach deliver gastric juice (containing HCl and enzymes, rennin and lipase) to each pit, and from there to the stomach lumen. There are about 5 million glands in the stomach, secreting some 2 liters of fluid per day.

Mucous-secreting cells are located on the inner surface of the stomach, in the pit and in the neck, the transitional region between pits and glands. These cells produce a thick layer of mucous which covers and protects the stomach, falling into 2 categories: the surface mucous is composed of neutral glycoproteins, while the mucous secreted by the neck mucous cells is composed of acidic glycoproteins. In cardiac and pyloric regions, mucous cells are also the major cell type in the glands.

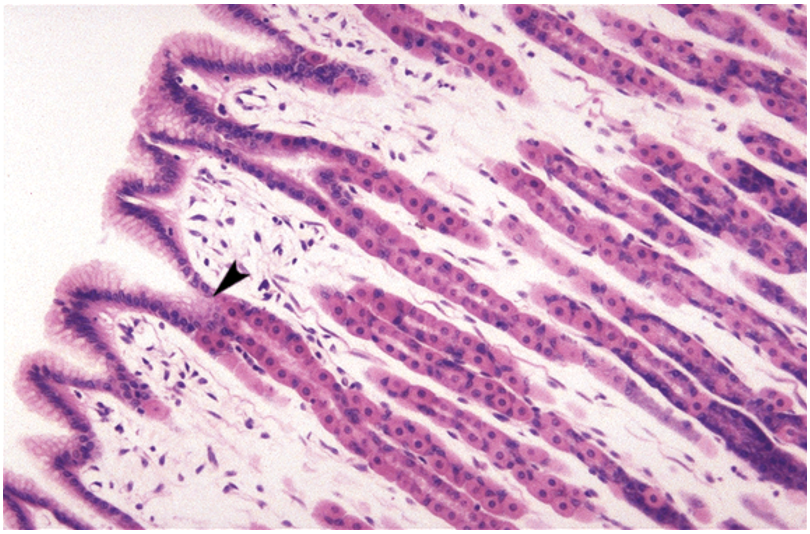

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Figure II-3-17. Cardiac part of the stomach with gastric pits and coiled gastric glands.

Stem cells that regenerate all stomach cells are located at the isthmus (arrowhead)

Oxyntic or parietal cells secrete 0.1N HCl into the stomach lumen and bicarbonate ions into the lamina propria (a byproduct of the acid production) in response to histamine, gastrin, and acetylcholine. These cells also secrete intrinsic factor (a glycoprotein necessary for absorption of vitamin B12). Vitamin B12 is required for production of erythrocytes; deficiencies result in pernicious anemia and a disruption of peripheral and central nervous system myelin (see subacute combined degeneration in *Neuroscience* section).

Parietal cells, located in the upper regions of the gastric glands, have a broader base and narrower apex. Their structure varies greatly depending on their functional state.

Clinical Correlate

Acetylcholine and gastrin increase HCL secretion by parietal cells. Histamine potentiates both by binding to the histamine H_2 receptor. Cimetidine and H_2 antagonists inhibit histamine.

Clinical Correlate

Infection by *Helicobacter pylori* affects the gastric mucosal lining and allows pepsin, HCl, and proteases to erode the mucosa.

Hematemesis and melena are common clinical findings.

Chief or **peptic cells** secrete pepsinogen, an enzyme precursor that is stored in secretory (zymogen) granules before its induced secretion. Pepsinogen is inactive and protects the peptic cells from autodigestion. Low pH, in the stomach lumen, converts pepsinogen to pepsin.

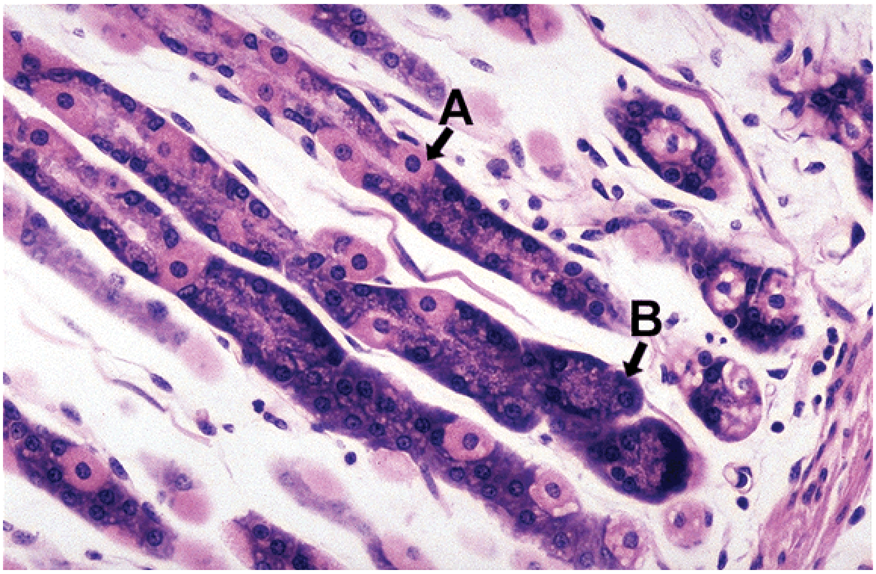

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Figure II-3-18. Stomach near the base of gastric glands

Pale-staining parietal cells (A) and chief cells (B) are shown.

The **enteroendocrine cells** or APUD cells (amine precursor uptake and decarboxylation) are present throughout the GI tract and are also found in the respiratory tract. They constitute a diffuse neuroendocrine system that collectively accounts for more cells than all other endocrine organs in the body. Enteroendocrine cells are dispersed throughout the GI tract so that they can receive and transmit local signals.

The **stem cells** responsible for the regeneration of all types of cells in the stomach epithelium are **located in the isthmus**. Their mitotic rate can be influenced by the presence of gastrin and by damage (aspirin, alcohol, bile salt reflux). Renewal of many gastric epithelial cells occurs every 4–7 days.

- Although the stem cells are capable of differentiating into any of the stomach cell types, there is evidence that the position of the cell along the gland influences its fate.
- In contrast to the short life span (4–5 days) of the cells near the acidic environment, the chief cells—deep within the glands—may have a life span >190 days.

Small Intestine

The small intestine is tubular in shape and has a total length of about 21 feet. The effective internal surface area of the small intestine is greatly increased by the plicae circulares, villi and microvilli.

Plicae circulares (circular folds or valves of Kerckring) are foldings of the inner surface that involve both mucosa and sub-mucosa. Plicae circulares increase the surface area by a factor of 3.

Villi arise above the muscularis mucosae and they include the lamina propria and epithelium of the mucosa. Villi increase the surface area by a factor of 10.

Microvilli of the absorptive epithelial cells increase the surface area by a factor of 20–30. The surface area of microvilli is increased even further by the presence of surface membrane glycoproteins, constituting the glycocalyx to which enzymes are bound.

The luminal surface of the small intestine is perforated by the openings of numerous tubular invaginations (the crypts of Lieberkühn) analogous to the glands of the stomach. The crypts penetrate through the lamina propria and reach the muscularis mucosae.

The small intestine completes digestion, absorbs the digested food constituents (amino acids, monosaccharides, fatty acids), and transports them into blood and lymphatic vessels.

The **duodenum** is the proximal pyloric end of the small intestine. Distal to the duodenum is the **jejunum**, and then the **ileum**.

In the small intestine, the chyme from the stomach is **mixed** with mucosal cell secretions, exocrine pancreatic juice, and bile.

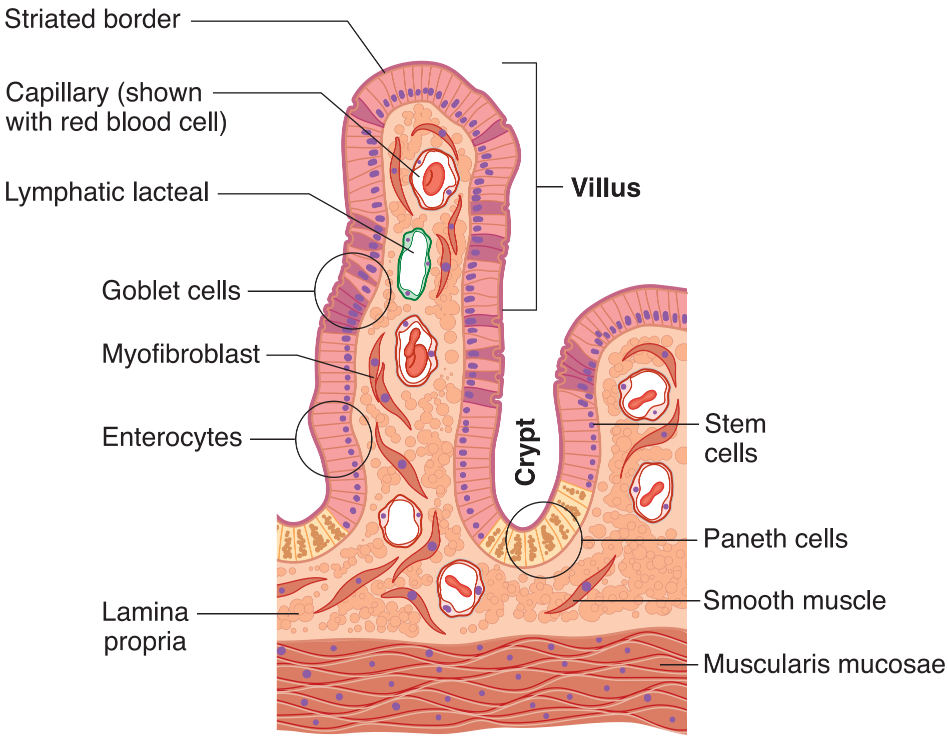

Figure II-3-19. Small Intestine Mucosal Histology

Clinical Correlate

Any compromise of the mucous protection can lead to significant damage and irritation of the gastrointestinal tract, leading to gastritis, duodenitis, or even peptic ulcer disease.

Clinical Correlate

Peristalsis is activated by the **parasympathetic system**. For those suffering from decreased intestinal motility manifesting as constipation (paralytic ileus, diabetic gastroparesis), dopaminergic and cholinergic agents are often used (e.g., metoclopramide).

Mucous production occurs in surface epithelial cells throughout the GI tract, by **Brunner glands** in the duodenum and **goblet cells** in the mucosa throughout the intestine.

Mucous functions include lubrication of the GI tract, binding bacteria, and trapping immunoglobulins where they have access to pathogens.

The **rate of mucous** secretion is increased by cholinergic stimulation, chemical irritation, and physical irritation.

In the **duodenum,** the acidic chyme from the stomach is neutralized by the neutral or alkaline mucous secretions of glands located in the submucosal or Brunner's glands. The duodenum also receives digestive enzymes and bicarbonate from the pancreas and bile from the liver (via gallbladder) through the bile duct, continuing the digestive process.

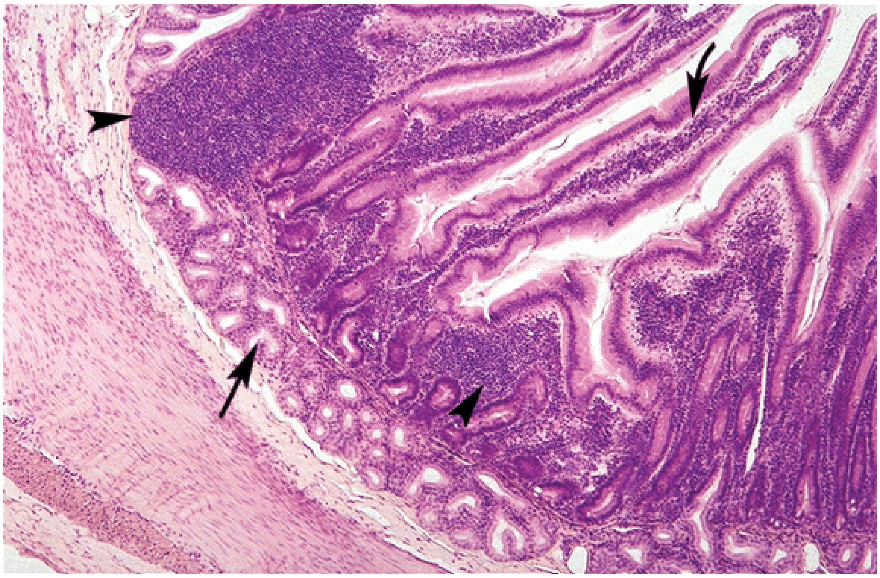

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Figure II-3-20. Duodenum with villi (curved arrow) and submucosal Brunner glands (arrow)

Patches of lymphatic tissue are in the lamina propria (arrowheads).

In the **jejunum,** the digestion process continues via enterocyte-produced enzymes and absorbs food products. The plicae circulares are best developed here. In the **ileum,** a major site of immune reactivity, the mucosa is more heavily infiltrated with lymphocytes and the accompanying antigen-presenting cells than the duodenum and jejunum. Numerous primary and secondary lymphatic nodules (Peyer's patches) are always present in the ileum's mucosa, though their location is not fixed in time. In the infant, maternal IgGs that are ingested are recognized by the Fc receptors in microvilli and endocytosed to provide passive immunity. In the adult, only trace amounts of intact proteins are transferred from lumen to lamina propria, but IgAs produced in GALT in the ileum are transported in the opposite direction into the lumen.

Throughout the small intestine, **the simple columnar intestinal epithelium has 5 types of differentiated cells, all derived from a common pool of stem cells.**

- **Goblet cells** secrete mucous that protects the surface of the intestine with a viscous fluid consisting of glycoproteins (20% peptides, 80% carbohydrates).
- **Enterocytes** have 2 major functions. Enterocytes participate in the final digestion steps and they absorb the digested food (in the form of amino acids, monosaccharides, and emulsified fats) by transporting it from the lumen of the intestine to the lamina propria, where it is carried away by blood vessels and lymphatics.
- **Paneth cells** are cells located at the base of the crypts, especially in the jejunum and ileum; they contain visible acidophilic secretory granules located in the apical region of the cells. These cells protect the body against pathogenic microorganisms by secreting lysozyme and defensins (or cryptins) that destroy bacteria. Their life span is about 20 days.

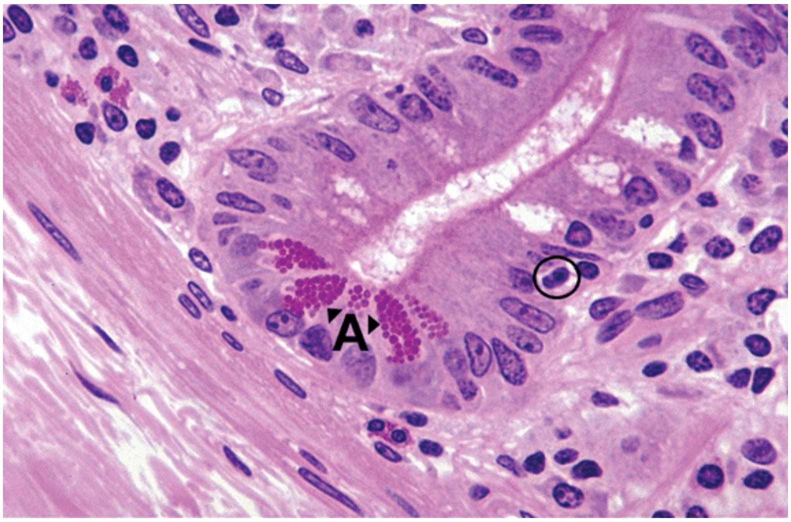

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Figure II-3-21. Cells at the base of crypts of Lieberkühn include Paneth cells (A) with large apical granules of lysozyme, and an adjacent stem cell (circle) undergoing mitosis.

- **Enteroendocrine cells** of the small and large intestine, like those of the stomach, secrete hormones that control the function of the GI tracts and associated organs. They are located in the lower half of the crypts and are detectable by silver-based stains.
- **Stem cells** are located in the crypts, about one-third of the way up from the bottom. Their progeny differentiates into all the other cell types. **The epithelial lining of the small intestine, particularly that covering the villi, completely renews itself every 5 days (or longer, during starvation).** The newly created cells (goblet, enterocytes, and enteroendocrine cells) migrate up from the crypts, while the cells at the tips of microvilli undergo apoptosis and slough off. There is also a

Note

Histologically, the duodenum contains submucosal Brunner's glands, and the ileum contains Peyer's patches in the lamina propria. The jejunum can be easily recognized because it has neither Brunner's glands nor Peyer's patches.

group of fibroblasts that accompany these epithelial cells as they move toward the tips of the villi. Other cells move to the base of the crypts, replenishing the population of Paneth and enteroendocrine cells.

Gut-associated lymphatic tissue (GALT): Throughout the intestine, the lamina propria is heavily infiltrated with macrophages and lymphocytes. Peyer's patches are patches of GALT that are prominent in the ileum. M cells in the epithelium transport luminal antigens to their base, where they are detected by B lymphocytes and taken up by antigen-presenting macrophages.

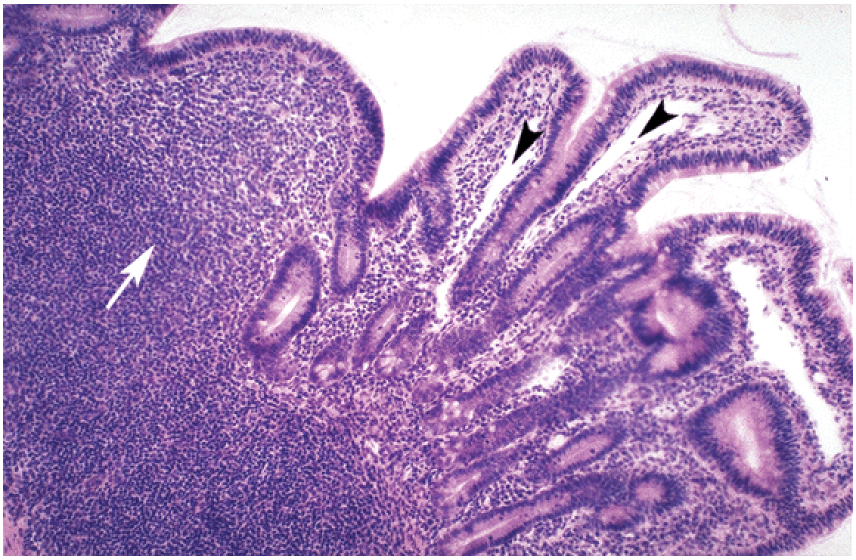

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Figure II-3-22. Ileum with Peyer's patches (arrow) and central lacteals (arrowheads) in the lamina propria of the villi

Large Intestine

The **large intestine** includes the cecum (with appendix), ascending, transverse, and descending colon, sigmoid colon, rectum, and anus. The large intestine has a wide lumen, strong musculature, and longitudinal muscle that is separated into 3 strands, the teniae coli. The inner surface has no plicae and no villi but consists of short crypts of Lieberkühn.

General features

- The colon is larger in diameter and shorter in length than is the small intestine. Fecal material moves from the **cecum**, through the colon (**ascending**, **transverse**, **descending**, and **sigmoid colons**), rectum, and anal canal.
- Three longitudinal bands of muscle, the **teniae coli**, constitute the outer layer. Because the colon is longer than these bands, pouching occurs, creating **haustra** between the teniae and giving the colon its characteristic "caterpillar" appearance.

- The mucosa has **no villi**, and mucous is secreted by short, inward-projecting colonic glands.
- Abundant lymphoid follicles are found in the cecum and appendix, and more sparsely elsewhere.
- The major functions of the colon are **reabsorption of fluid and electrolytes** and **temporary storage of feces**.

Defecation

Rectal distention with feces activates intrinsic and cord reflexes that cause relaxation of the internal anal sphincter (smooth muscle) and produce the urge to defecate. If the external anal sphincter (skeletal muscle innervated by the **pudendal nerve**) is then voluntarily relaxed, and intra-abdominal pressure is increased via the **Valsalva maneuver**, defecation occurs. If the external sphincter is held contracted, the urge to defecate temporarily diminishes.

Throughout the large intestine, the epithelium contains goblet, absorptive, and enteroendocrine cells. Unlike the small intestine, about half of the epithelial cells are mucous-secreting goblet cells, providing lubrication. The major function of the large intestine is fluid retrieval. Some digestion is still occurring, mainly the breakdown of cellulose by the permanent bacterial flora. Stem cells are in the lower part of the crypts.

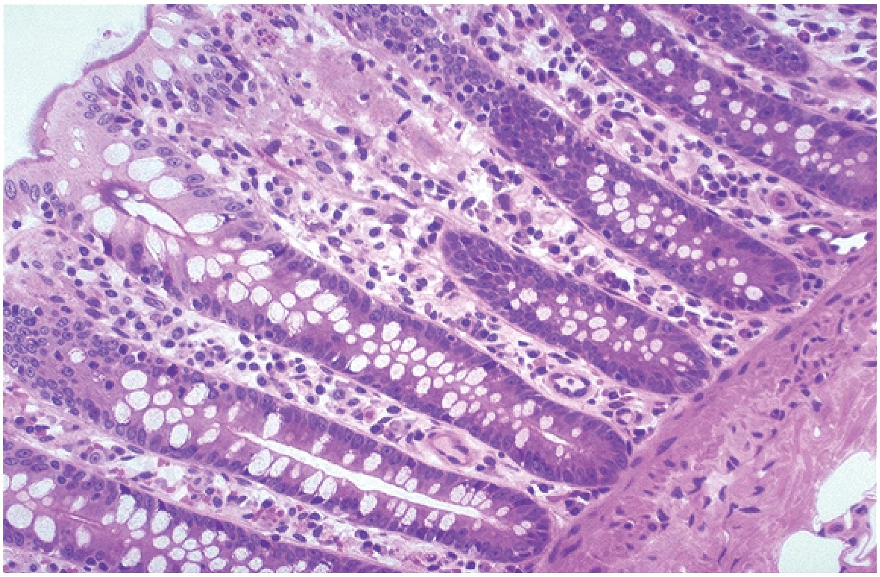

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Figure II-3-23. Large intestine with crypts but no villi and many light-staining goblet cells interspersed among enterocytes

GASTROINTESTINAL GLANDS

Salivary Glands

The major salivary glands are all branched tubuloalveolar glands, with secretory **acini** that drain into **ducts**, which drain into the oral cavity. Acini contain **serous**, **mucous**, or both types of secretory cells, as well as **myoepithelial cells**, both surrounded by a basal lamina. Serous cells secrete various proteins and enzymes. Mucous cells secrete predominantly glycosylated mucins.

Table II-3-5. Gastrointestinal Glands

Salivary glands Submandibular Parotid Sublingual	• Produce approximately 1.5 L/day of saliva • Presence of food in the mouth; the taste, smell, sight, or thought of food; or the stimulation of vagal afferents at the distal end of the esophagus increase production of saliva	
Functions	• Initial triglyceride digestion (lingual lipase) • Initial starch digestion (α-amylase) • Lubrication	
Regulation	**Parasympathetic**	↑ synthesis and secretion of **watery** saliva via muscarinic receptor stimulation; (anticholinergics → dry mouth)
	Sympathetic	↑ synthesis and secretion of **viscous** saliva via β-adrenergic receptor stimulation

The ducts that drain the glands increase in size and are lined by an epithelium that transitions from cuboidal to columnar to pseudostratified to stratified columnar cells. The smallest ducts, **intercalated ducts**, have myoepithelial cells; the next larger ducts, **striated ducts**, have columnar cells with basal striations, caused by basal infolding of cell membranes between prominent mitochondria. These columnar cells make the saliva hypotonic by transporting Na and Cl ions out of saliva back into the blood.

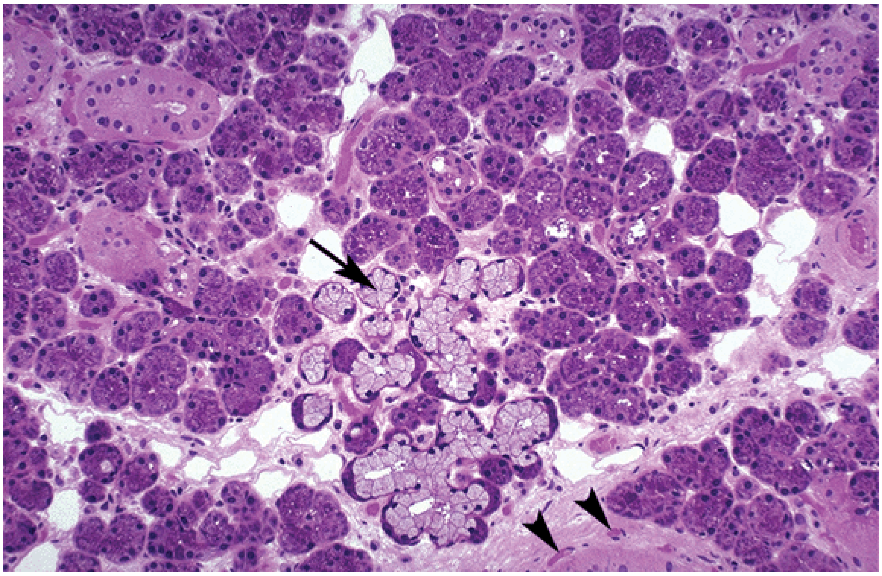

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Figure II-3-24. Submandibular with a mix of light-staining mucous acini (arrow) adjacent to dark-staining serous acini

Small vessels (arrowheads)

- The **parotid glands** lie on the surface of the masseter muscles in the lateral face, in front of each external auditory meatus. The parotids are **entirely serous** salivary glands that drain inside each cheek through **Stensen's ducts** which open above the second upper molar tooth. The parotid glands contribute 25% of the volume of saliva.
- The **submandibular glands** lie inside the lower edge of the mandible, and are mixed serous/mucous glands with a **predominance of serous** cells. They drain in the floor of the mouth near the base of the tongue through **Wharton's ducts**. The submandibular glands contribute 70% of the volume of saliva.
- The **sublingual glands** lie at the base of the tongue, and are also mixed serous/mucous glands with a **predominance of mucous** cells. They drain into the mouth through multiple small ducts. The sublingual glands contribute 5% of the volume of saliva.

Clinical Correlate

The parotid gland is the major site of the mumps and rabies viruses that are transmitted in saliva.

Benign tumors most frequently appear at the parotid gland; their removal is complicated by the facial nerve traversing the gland.

Exocrine Pancreas

The pancreas is a branched tubuloacinar exocrine gland with **acini**. The acini are composed of secretory cells that produce multiple digestive enzymes including proteases, lipases, and amylases. Acinar cells are functionally polarized, with basophilic RER at their basal ends below the nucleus, and membrane-bound, enzyme-containing eosinophilic **zymogen granules** toward their apex.

The endocrine-producing cells of the **islets of Langerhans** are embedded within the exocrine pancreas.

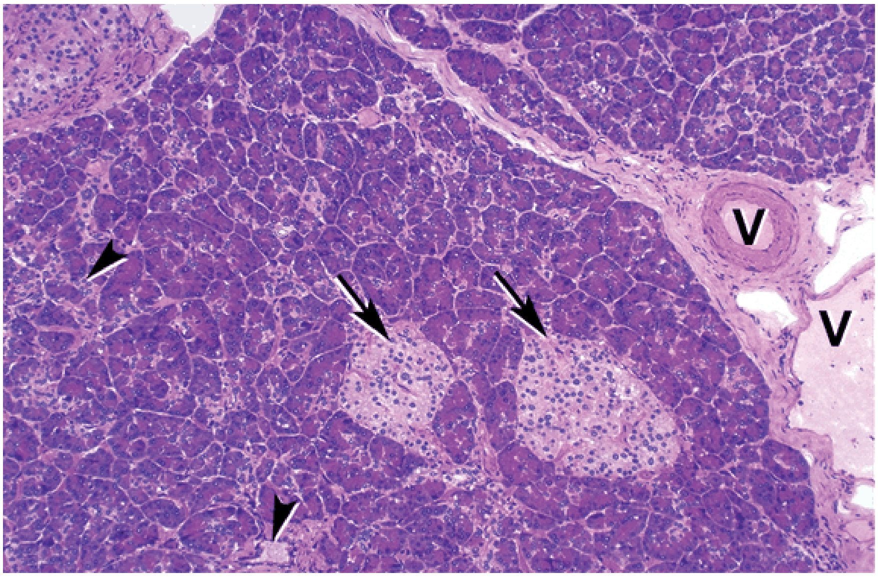

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Figure II-3-25. Pancreas with light-staining islets of Langerhans (arrows) surrounded by exocrine acini with ducts (arrowheads) and adjacent blood vessels (V)

Unlike salivary glands, the **pancreas lacks myoepithelial cells** in acini and **lacks striated ducts**. Also, unlike salivary glands, the cells of the intercalated ducts extend partially into the lumen of pancreatic acini as **centroacinar cells**. The pancreas does not usually have mucinous cells in acini, but may have mucinous cells in its ducts.

Pancreatic acini drain via progressively larger ducts into the duodenum. The main pancreatic **duct (of Wirsung)** is the distal portion of the dorsal pancreatic duct that joined the ventral pancreatic duct in the head of the pancreas. The main duct typically joins with the common bile duct and enters the duodenum through the **ampulla of Vater** (controlled by the **sphincter of Oddi**). Sometimes the pancreas has a persistent accessory duct with separate drainage into the duodenum, the **accessory duct of Santorini**, and a persisting remnant of the proximal part of the dorsal pancreatic duct.

Liver

The liver is the largest visceral organ and gland (both exocrine and endocrine) in the body. **Hepatocytes**, of endodermal epithelial origin, carry out both exocrine and endocrine functions. The liver has unusual capillaries, the hepatic **sinusoids**, that facilitate exchange (uptake and secretion) between hepatocytes and blood. The liver has **Kupffer cells**, specialized cells of the mononuclear phagocyte system (blood monocyte derived) which patrol the **space of Disse** between hepatocytes and blood.

The liver has epithelial lined exocrine (excretory) ducts, the **bile ducts**, lined by **biliary epithelium**, which drain hepatocyte products (bile) into the duodenum, ultimately through the common bile duct. Blood flow into the liver is dual (75% from **portal vein**, 25% from **hepatic artery**), while blood flow out is via **hepatic veins** into the inferior vena cava.

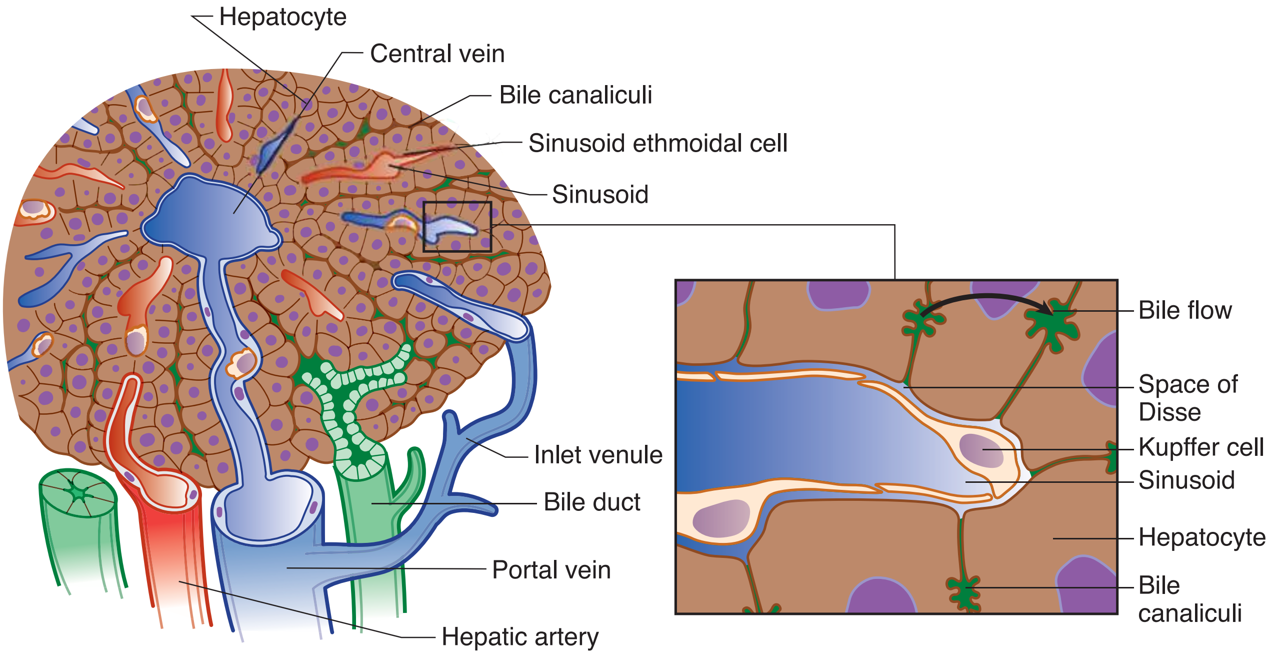

Figure II-3-26. Organization of a Liver Lobule

Hepatocytes are functionally polarized, like many other epithelial cells, but rather than being polarized along a single axis, each hepatocyte has multiple "**basal**" and "**apical**" surfaces.

- Where 2 hepatocytes abut, their "apical" surfaces form bile **canaliculi**, extracellular grooved bile channels joined by tight and occluding junctions between adjacent hepatocytes. Bile is secreted into bile canaliculi, which drains into progressively larger bile ducts and empties into the duodenum.
- Where a hepatocyte abuts a sinusoid, its membrane has microvilli, representing a "basal" or basolateral surface. Exchange between blood and hepatocytes is facilitated by the surface microvilli. This exchange occurs in the space of Disse, which is between the fenestrated endothelial cells of the sinusoid and the basal surface of hepatocytes.

The hepatic artery and portal vein enter and the common hepatic duct exits the liver in the hepatic **hilum**. Within the liver, branches of the hepatic artery, portal vein, and bile duct tend to run together in thin connective tissue bands. When seen in cross section, these 3 structures and their connective tissue are called a **portal triad or portal tract**. Blood from both the portal vein and hepatic artery branches flows through and mixes in hepatic sinusoids that run between cords or plates of hepatocytes. After passing by hepatocytes, the sinusoidal blood flows into hepatic venules, which form progressively larger branches draining into the right and left hepatic veins which drain into the inferior vena cava.

A **classic hepatic lobule** is a hexagonal structure with a portal tract at each corner of the hexagon and a central vein in the center of the hexagon. Blood flow is from the triads into the central vein and bile flow is opposite, from the central vein to the triads.

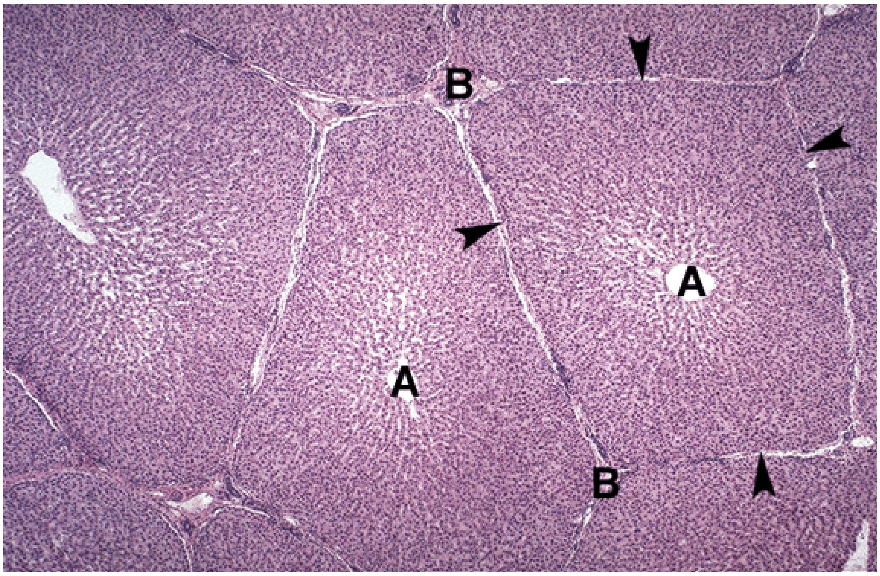

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Figure II-3-27. Liver lobules with central veins (A) in the center of each lobule and connective tissue (arrowheads) separating each lobule and portal triads at each point of the lobule (B)

A **portal lobule** is a triangular structure with a central vein at each corner and a portal tract in the center. Bile flows from the periphery of the portal lobule into the central triad.

A **hepatic acinus** is based on blood flow from the hepatic artery branches to central veins. As hepatic arterial blood flow enters the sinusoids from side branches extending away from the center of the hepatic triad (rather than directly from the triad), the center of the acinus is conceived of as centered on such a branch extending out from a triad (or between 2 triads) and ending at 2 nearby central veins, resulting in a roughly elliptical structure with portal tracts at the 2 furthest poles and 2 central veins at the 2 closest edges.

In the acinus, the hepatocytes receiving the first blood flow (and the most oxygen and nutrients) are designated **zone 1**, while those receiving the last blood flow (and least oxygen and nutrients) are near the central veins and designated **zone 3**. **Zone 2** hepatocytes are in between zones 1 and 3. This model helps to explain the **differential effect on hepatocytes of changes in blood flow, oxygenation, etc**. Zone 3 is most susceptible to injury by decreased oxygenation of blood or decreased blood flow into the liver (as well as stagnation of blood drainage out of the liver due to congestive heart failure).

The metabolic activity of hepatocytes varies within the zones of the acinus. Zone 1 hepatocytes are most involved in glycogen synthesis and plasma protein synthesis (albumin, coagulation factors and complement components). Zone 3 cells are most concerned with lipid, drug, and alcohol metabolism and detoxification.

Ito cells (stellate cells) are mesenchymal cells that live in the space of Disse. They contain fat and are involved in **storage of fat-soluble vitamins, mainly vitamin A**.

Bile formation by hepatocytes serves both an exocrine and excretory function. Bile salts secreted into the duodenum aid in fat emulsification and absorption, as well as excretion of endogenous metabolites (bilirubin) and drug metabolites that cannot be excreted by the kidney.

Gallbladder

The gallbladder is lined by a simple columnar epithelium with both absorptive and mucin secretory function, with underlying lamina propria. Unlike the gut tube, the gallbladder **lacks muscularis mucosae and submucosa**, and the muscularis externa is not organized into 2 distinct layers like the gut. The surface of the gallbladder is covered by peritoneal serosa (mesothelium). The gallbladder has a wide end, the **fundus**, and a narrowing **neck**, which empties into the **cystic duct** and which has spiral valves of Heister. The cystic duct joins the **common hepatic duct** to form the **common bile duct**, which joins the pancreatic duct at or just before the ampulla of Vater.

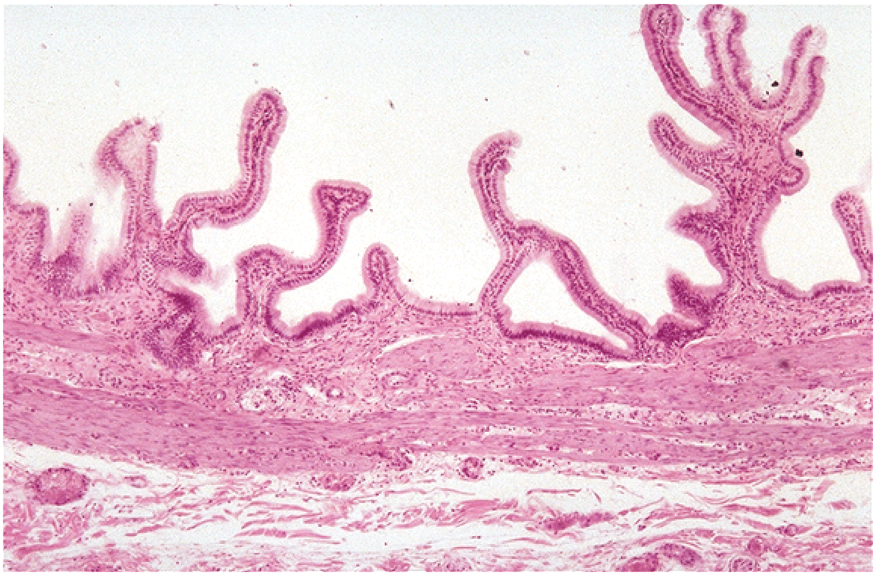

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Figure II-3-28. Gallbladder with folds lined by a simple columnar epithelium with no goblet cells

The gallbladder stores and concentrates bile by absorption of electrolytes and water. After a meal, entrance of lipid into the duodenum stimulates enteroendocrine cells of the duodenum to secrete cholecystokinin, which stimulates contraction and emptying of the gallbladder. At the same time, it relaxes the sphincter of Oddi in the ampulla of Vater. This delivers a bolus of bile into the duodenum.

Clinical Correlate

When stimulated during liver injury, Ito cells may release type I collagen and other matrix components into the space of Disse, **contributing to scarring** of the liver in some diseases (cirrhosis due to ethanol). This may lead to the development of portal hypertension, portacaval anastomoses, and esophageal or rectal bleeding.

Clinical Correlate

Disturbance of the balance in the components of bile can lead to precipitation of one or more of the bile components, resulting in stone (or **calculus**) formation or **lithiasis** in the gallbladder and/or bile ducts.

Note

Main Functions of Bile

- Absorption of fats from intestinal lumen
- Excretion
- Transport of IgA

Note

Abdominal Aorta Branches

Visceral branches

Unpaired: celiac (foregut), superior mesenteric (midgut), inferior mesenteric (hindgut)

Paired: middle suprarenals, renals, gonadals

Parietal branches

Unpaired: median sacral

Paired: inferior phrenics, lumbars, common iliac

Clinical Correlate

The most common site for an **abdominal aneurysm** is in the area between the renal arteries and the bifurcation of the abdominal aorta. Signs include decreased circulation to the lower limbs and pain radiating down the back of the lower limbs. The most common site of **atherosclerotic plaque** is at the bifurcation of the abdominal aorta.

ARTERIAL SUPPLY TO ABDOMINAL VISCERA

The blood supply to the abdominal viscera and the body wall is provided by branches of the abdominal aorta. The aorta enters the abdomen by passing through the aortic hiatus of the diaphragm at the **T12 vertebra** (Figure II-3-28). It descends on the lumbar vertebra just to the left of the midline and bifurcates at the **L4 vertebral** level. During its short course in the abdomen, the aorta gives origin to 3 groups of branches: (a) **3 unpaired visceral branches**, (b) **3 paired visceral branches**, and (c) several **parietal branches** to the body wall.

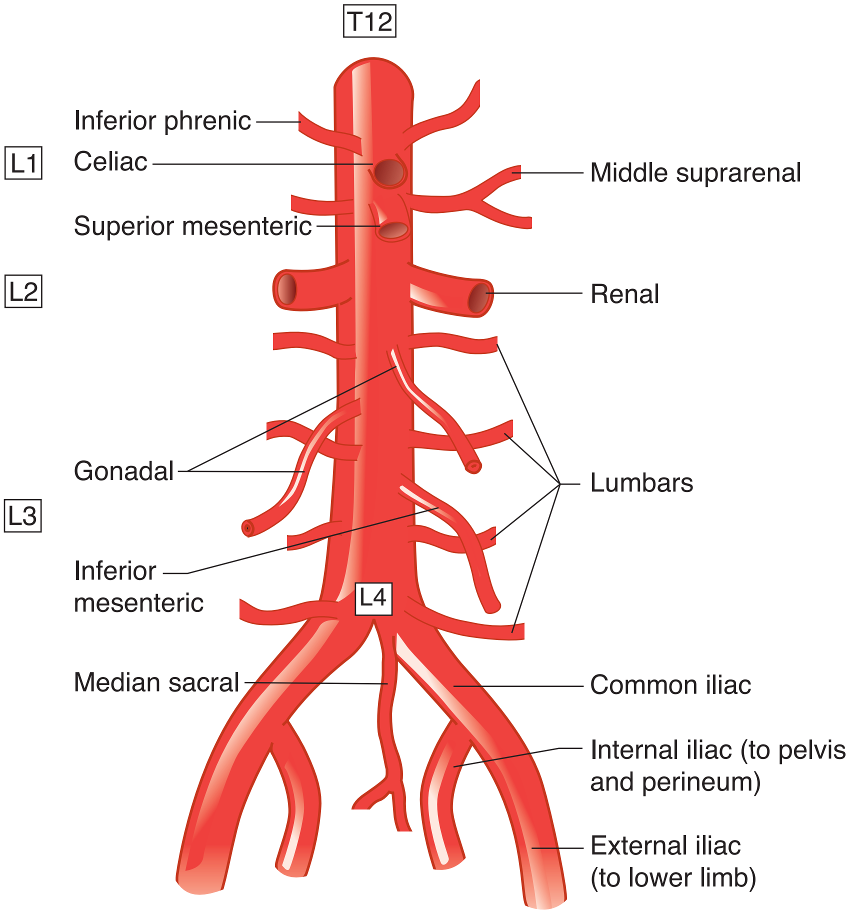

Figure II-3-29. Visceral and Parietal Branches of the Abdominal Aorta

Three Unpaired Visceral Arteries

Celiac Artery (Trunk)

The **celiac artery** is the blood supply to the structures derived from the foregut. The artery arises from the anterior surface of the aorta just inferior to the aortic hiatus at the level of T12–L1 vertebra. The celiac artery passes above the superior border of the pancreas and then divides into 3 retroperitoneal branches.

The **left gastric artery** courses superiorly and upward to the left to reach the **lesser curvature** of the stomach. The artery enters the lesser omentum and

follows the lesser curvature distally to the pylorus. The distribution of the left gastric includes the following:

- **Esophageal branch** to the distal one inch of the esophagus in the abdomen
- **Most of the lesser curvature**

The **splenic artery** is the longest branch of the celiac trunk and runs a very tortuous course along the superior border of the pancreas. The artery is retroperitoneal until it reaches the tail of the pancreas, where it enters the splenorenal ligament to enter the hilum of the spleen. The distributions of the splenic artery include:

- **Direct branches to the spleen**
- **Direct branches to the neck, body, and tail of pancreas**
- **Left gastroepiploic artery** that supplies the left side of the **greater curvature** of the stomach
- **Short gastric** branches that supply to the **fundus** of the stomach

The **common hepatic artery** passes to the right to reach the superior surface of the first part of the duodenum, where it divides into its 2 terminal branches:

- **Proper hepatic artery** ascends within the hepatoduodenal ligament of the lesser omentum to reach the porta hepatis, where it divides into the **right and left hepatic arteries**. The right and left arteries enter the 2 lobes of the liver, with the right hepatic artery first giving rise to the **cystic artery** to the gallbladder.
- **Gastroduodenal artery** descends posterior to the first part of the duodenum and divides into the **right gastroepiploic artery** (supplies the pyloric end of the greater curvature of the stomach) and the **superior pancreaticoduodenal arteries** (supplies the head of the pancreas, where it anastomoses the inferior pancreaticoduodenal branches of the superior mesenteric artery).

Clinical Correlate

- The **splenic artery** may be subject to erosion by a penetrating ulcer of **the posterior wall** of the stomach into the lesser sac.
- The **left gastric artery** may be subject to erosion by a penetrating ulcer of the **lesser curvature** of the stomach.
- The **gastroduodenal artery** may be subject to erosion by a penetrating ulcer of the **posterior wall** of the first part of the duodenum.

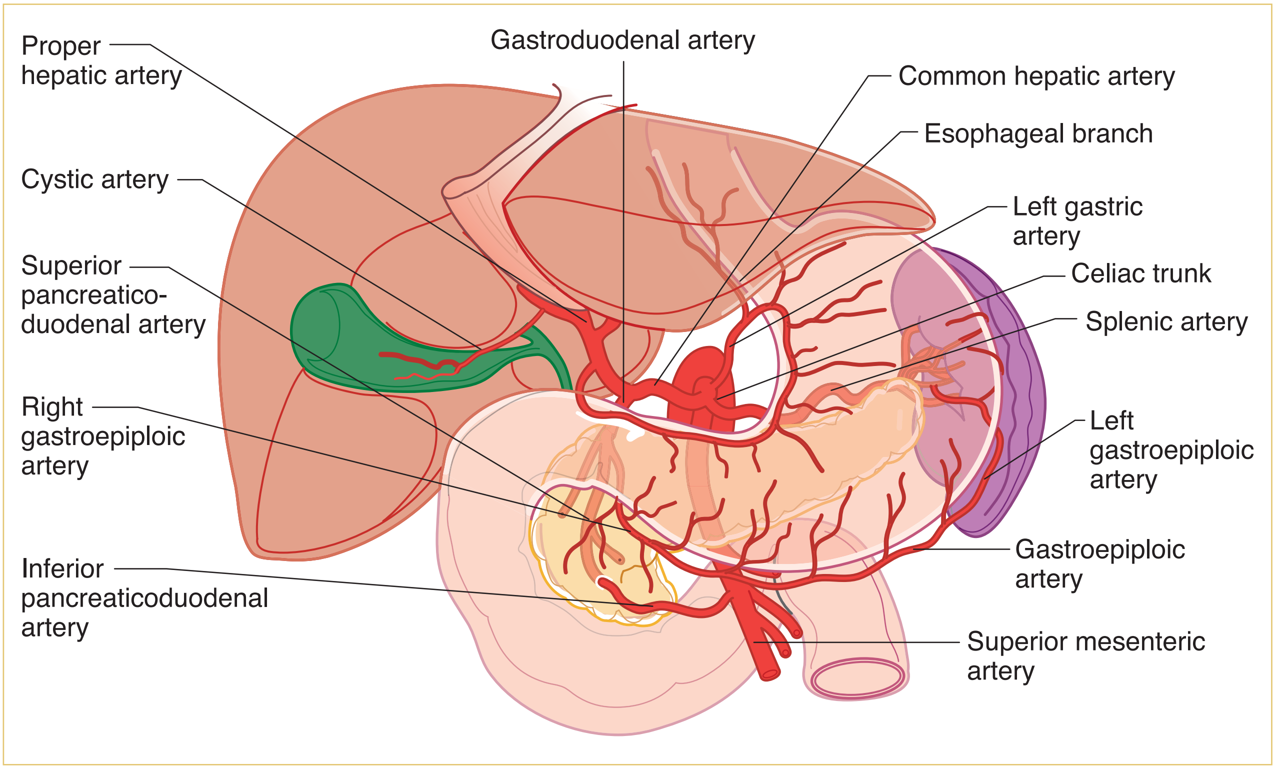

Figure II-3-30. Celiac Artery

Superior Mesenteric Artery

The **superior mesenteric artery** (SMA) supplies the viscera of the midgut. The SMA arises from the aorta deep to the neck of the pancreas just below the origin of the celiac artery at the L1 vertebral level. It then descends anterior to the uncinate process of the pancreas and the third part of the duodenum to enter the mesentery proper. The superior mesenteric vein is to the right of the artery. Branches of the SMA include:

- **Inferior pancreaticoduodenal arteries** which anastomose with the superior pancreaticoduodenal branches of the gastroduodenal artery in the head of the pancreas
- **Intestinal arteries** arise as 12–15 branches from the left side of the SMA and segmentally supply the jejunum and ileum. Distally, they form vascular arcades and vasa recta arteries at the wall of the gut.
- **Ileocolic artery** is the most inferior branch which descends to the lower right quadrant to supply the distal ileum and cecum.
- **Right colic artery** passes to the right to supply the ascending colon.
- **Middle colic artery** ascends and enters the transverse mesocolon to supply the proximal two-thirds of the transverse colon.

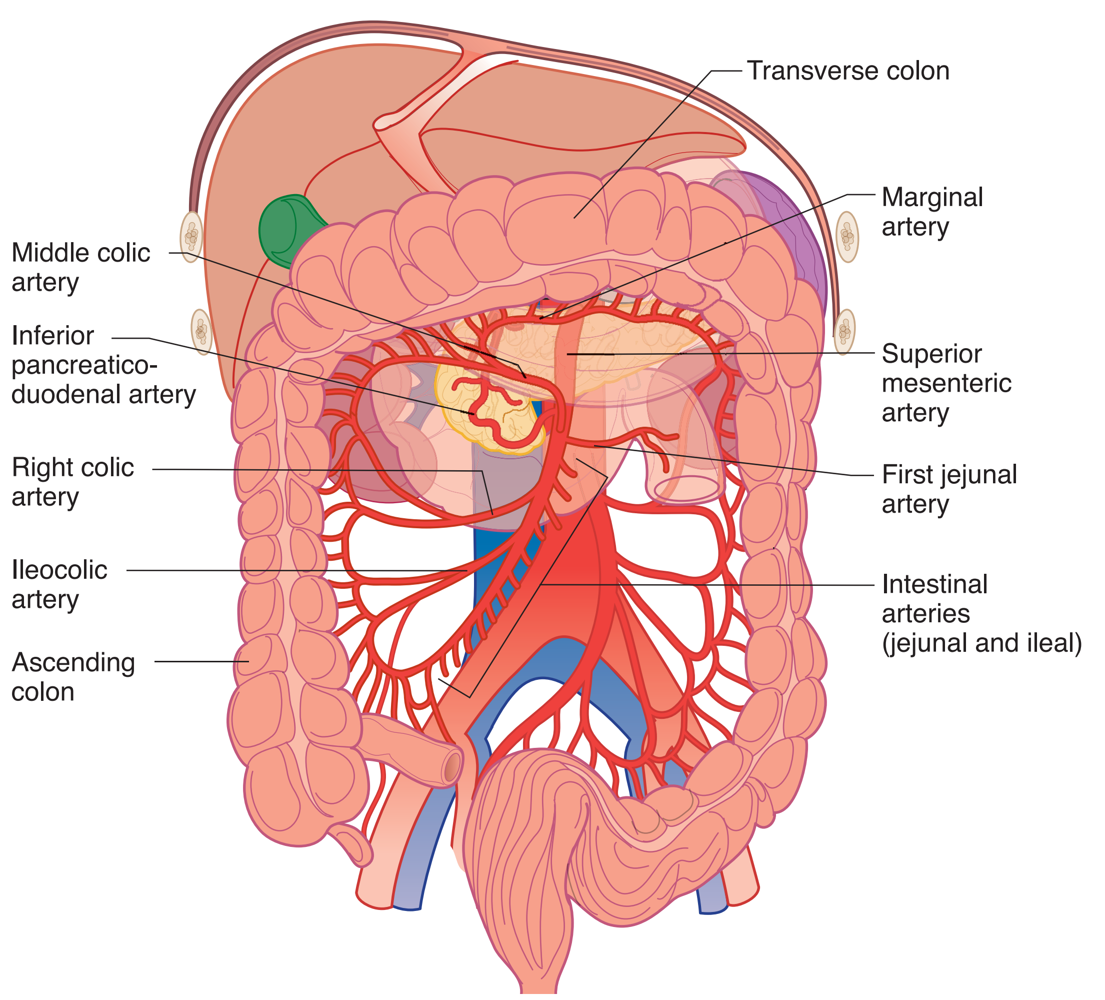

Figure II-3-31. Distribution of Superior Mesenteric Artery

Inferior Mesenteric Artery

The inferior mesenteric artery (IMA) is the third unpaired visceral branch of the aorta that supplies the hindgut (distal third of the transverse colon to the pectinate line). It arises from the aorta just above its bifurcation at the level of the L3 vertebra. It descends retroperitoneally and inferiorly to the left and gives rise to 3 branches:

- **Left colic artery** supplies the distal third of the transverse colon and the descending colon
- **Sigmoid arteries** to the sigmoid colon
- **Superior rectal artery** descends into the pelvis and supplies the superior aspect of the rectum and anal canal.

The branches of the SMA and IMA to the ascending, transverse and descending parts of the large intestines are interconnected by a continual arterial arch called the **marginal artery.** The marginal artery provides a collateral circulation between the parts of the large intestines if there is a vascular obstruction in some part of the SMA and IMA.

Clinical Correlate

Branches of the **celiac** and **superior mesenteric arteries** form a collateral circulation within the head of the pancreas.

Clinical Correlate

The **splenic flexure** is the most common site of bowel ischemia.

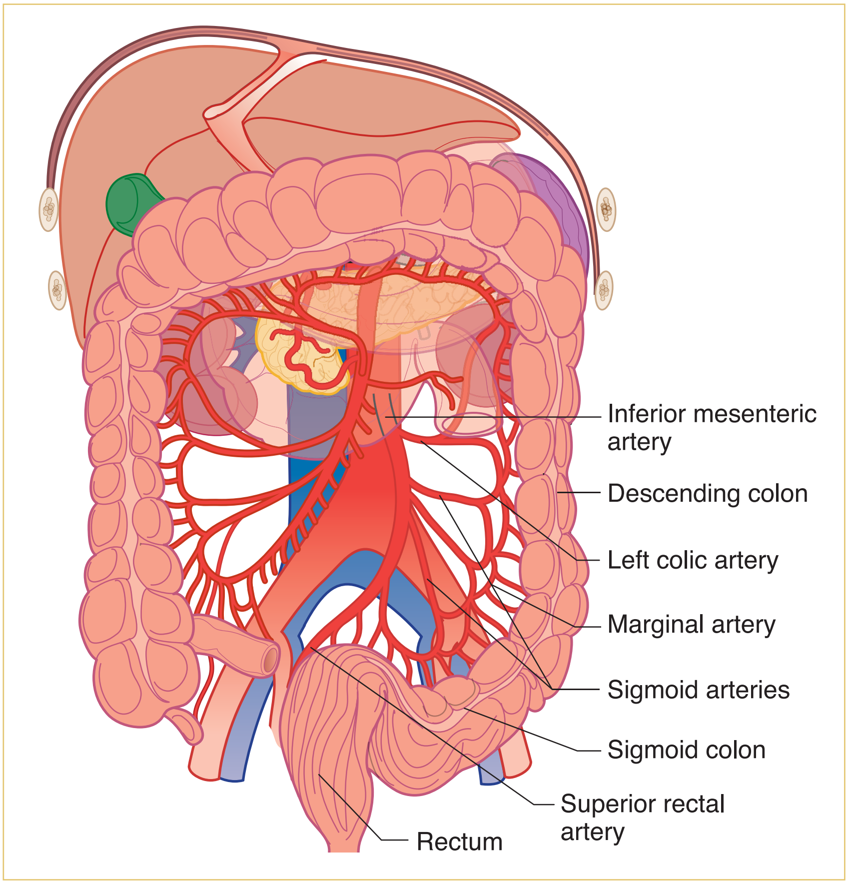

Figure II-3-32. Distribution of Inferior Mesenteric Artery

Clinical Correlate

The **left renal vein may be compressed** by an **aneurysm of the superior mesenteric artery** as the vein crosses anterior to the aorta. Patients with compression of the left renal vein may have renal and adrenal hypertension on the left, and, in males, a varicocele on the left.

Three Paired Visceral Arteries

- **Middle suprarenal arteries** branch from the aorta above the renal arteries and supply the medial parts of the suprarenal gland.
- **Renal arteries** are large paired vessels that arise from the aorta at the upper border of the L2 vertebra. They course horizontally to the hila of the kidneys. The right renal artery is the longer than the left and passes posterior to the inferior vena cava.
- **Gonadal arteries** arise from the anterior surface of the aorta just inferior to the renal arteries. They descend retroperitoneally on the ventral surface of the psoas major muscle.

VENOUS DRAINAGE OF ABDOMINAL VISCERA

Inferior Vena Cava

The **inferior vena cava** forms to the right of the lumbar vertebrae and the abdominal aorta by the union of the 2 common iliac veins at the **L5 vertebral level**. The inferior vena cava ascends to the right of the midline and passes through the caval hiatus of the diaphragm at the **T8 vertebral level**. The inferior vena cava receives blood from the lower limbs, pelvis and perineum,

paired abdominal viscera, and body wall. Note that the vena cava does not receive blood directly from the GI tract, except the lower rectum and anal canal.

The **right tributaries** (right gonadal, right suprarenal) drain separately into the inferior vena cava. But on the left side, the left gonadal and left suprarenal veins drain into the left renal vein, which then drains into the vena cava. The left renal vein crosses anterior to the aorta, just inferior to the origin of the superior mesenteric artery.

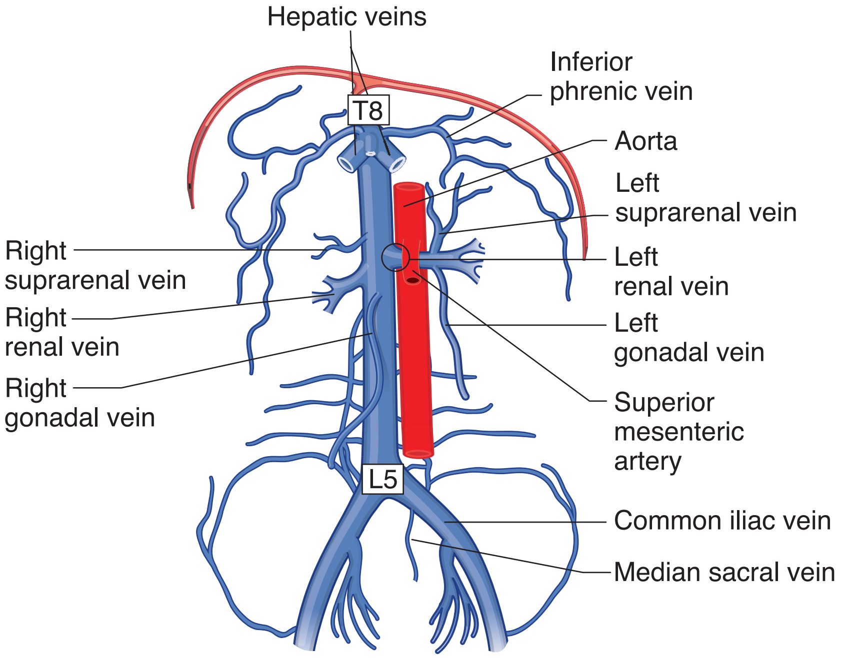

Figure II-3-33. Inferior Vena Cava and Tributaries

Hepatic Portal System

The **hepatic portal system** is an extensive network of veins that receives the blood flow from the GI tract above the pectinate line. The venous flow is carried to the liver via the **hepatic portal vein** where it enters the liver sinusoids, which drain to the hepatic veins, which then drain into the inferior vena cava and ultimately into the right atrium.

The hepatic portal vein is formed by the union of the **superior mesenteric** (drains midgut) and **splenic** (drains foregut) veins posterior to the **neck of the pancreas**. **The inferior mesenteric vein** (drains hindgut) usually drains into splenic vein.

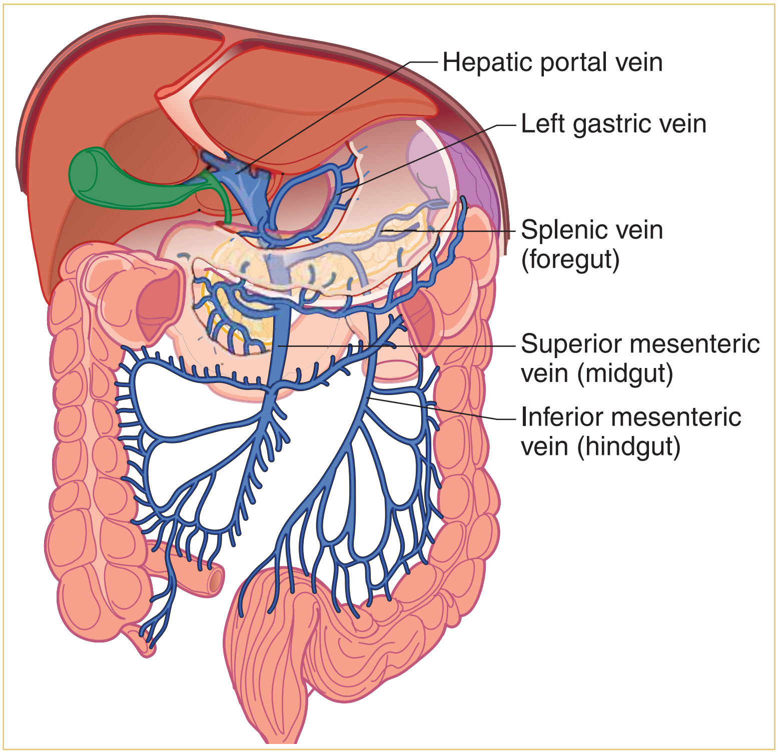

Figure II-3-34A. Hepatic Portal System

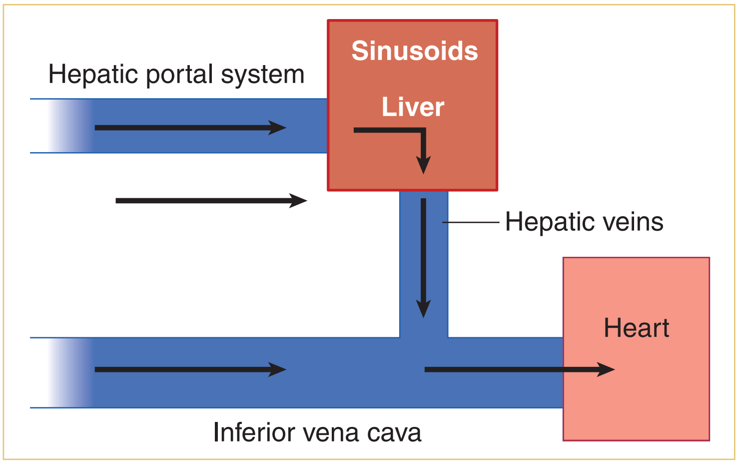

Figure II-3-34B. Comparison of Normal Caval and Portal Blood Flow

If there is an obstruction to flow through the portal system **(portal hypertension)**, blood can flow in a retrograde direction (because of the absence of valves in the portal system) and pass through **anastomoses** to reach the caval system. Sites for these anastomoses include the **esophageal veins, rectal veins**, and **thoracoepigastric veins**. Enlargement of these veins may result in esophageal varices, hemorrhoids, or a caput medusae.

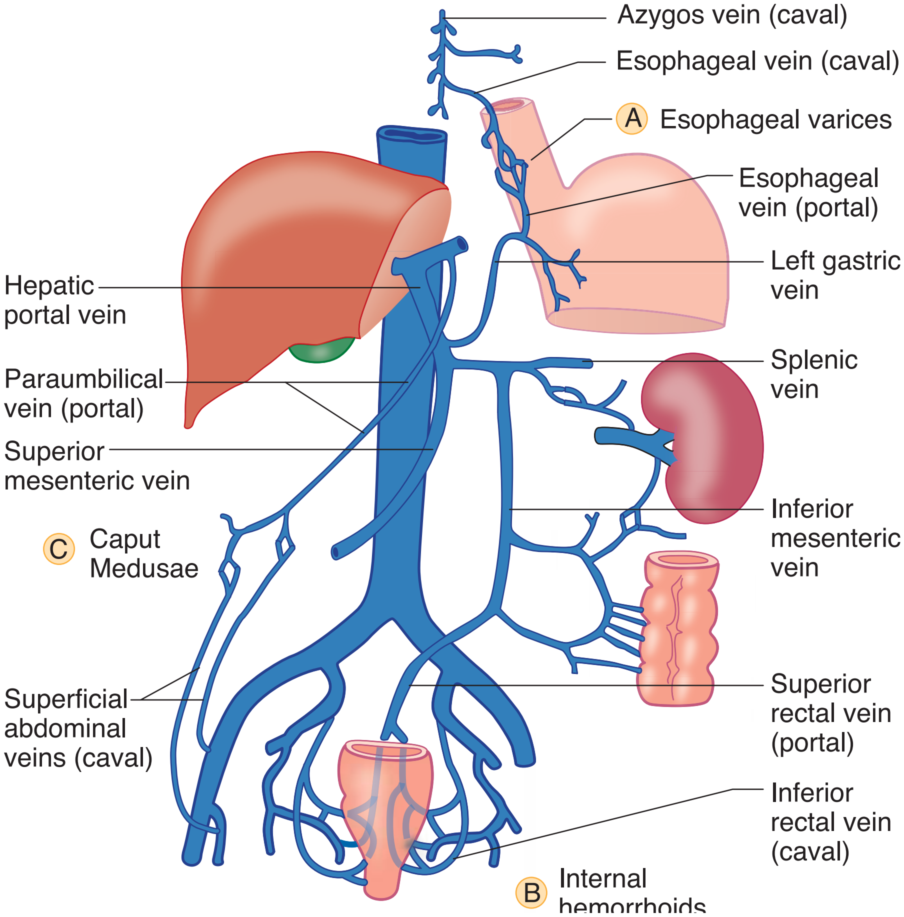

Figure II-3-35. Chief Portacaval Anastomoses

Table II-3-6. Sites of Portacaval Anastomoses

Sites of Anastomoses	Portal		Caval	Clinical Signs
Esophagus A	Esophageal veins (left gastric veins)	→	Veins of the thoracic esophagus, which drain into the azygos system	Esophageal varices
Rectum B	Superior rectal veins (inferior mesenteric vein)	→	Inferior rectal veins (internal iliac vein)	Internal hemorrhoids
Umbilicus C	Paraumbilical veins	→	Superficial veins of the anterior abdominal wall	Caput medusa

POSTERIOR ABDOMINAL BODY WALL

Embryology of Kidneys and Ureter

Renal development is characterized by 3 successive, slightly overlapping kidney systems: **pronephros**, **mesonephros**, and **metanephros**.

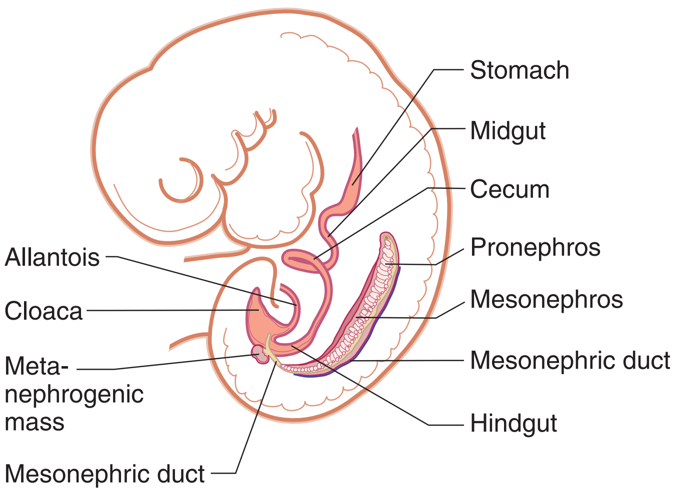

Figure II-3-36. Pronephros, Mesonephros, and Metanephros

During week 4, segmented nephrotomes appear in the cervical intermediate mesoderm of the embryo. These structures grow laterally and canalize to form nephric tubules. The first tubules formed regress before the last ones are formed. By the end of week 4, the pronephros disappears and does not function.

In week 5, the mesonephros appears as S-shaped tubules in the intermediate mesoderm of the thoracic and lumbar regions of the embryo.

- The medial end of each tubule enlarges to form a Bowman's capsule into which a tuft of capillaries, or glomerulus, invaginates.
- The lateral end of each tubule opens into the **mesonephric (Wolffian) duct**, an intermediate mesoderm derivative. The duct drains into the hindgut.
- Mesonephric tubules function temporarily and degenerate by the beginning of month 3. The mesonephric duct persists in the male as the ductus epididymidis, ductus deferens, and the ejaculatory duct. It disappears in the female.

During week 5, the metanephros, or permanent kidney, develops from 2 sources: the **ureteric bud**, a diverticulum of the mesonephric duct, and the **metanephric mass** (blastema), from intermediate mesoderm of the lumbar and sacral regions.

The **ureteric bud** penetrates the metanephric mass, which condenses around the diverticulum to form the metanephrogenic cap. The bud dilates to form the renal pelvis, which subsequently splits into the cranial and caudal major calyces. Each major calyx buds into the metanephric tissue to form the minor calyces.

One to 3 million collecting tubules develop from the minor calyces, thus forming the renal pyramids. The ureteric bud forms the **drainage components** of the urinary system (calyces, pelvis, ureter).

Penetration of collecting tubules into the **metanephric mass** induces cells of the tissue cap to form **nephrons**, or excretory units. Lengthening of the excretory tubule gives rise to the proximal convoluted tubule, the loop of Henle, and the distal convoluted tubule.

The kidneys develop in the pelvis but appear to ascend into the abdomen as a result of fetal growth of the lumbar and sacral regions. With their ascent, the ureters elongate, and the kidneys become vascularized by arteries which arise from the abdominal aorta.

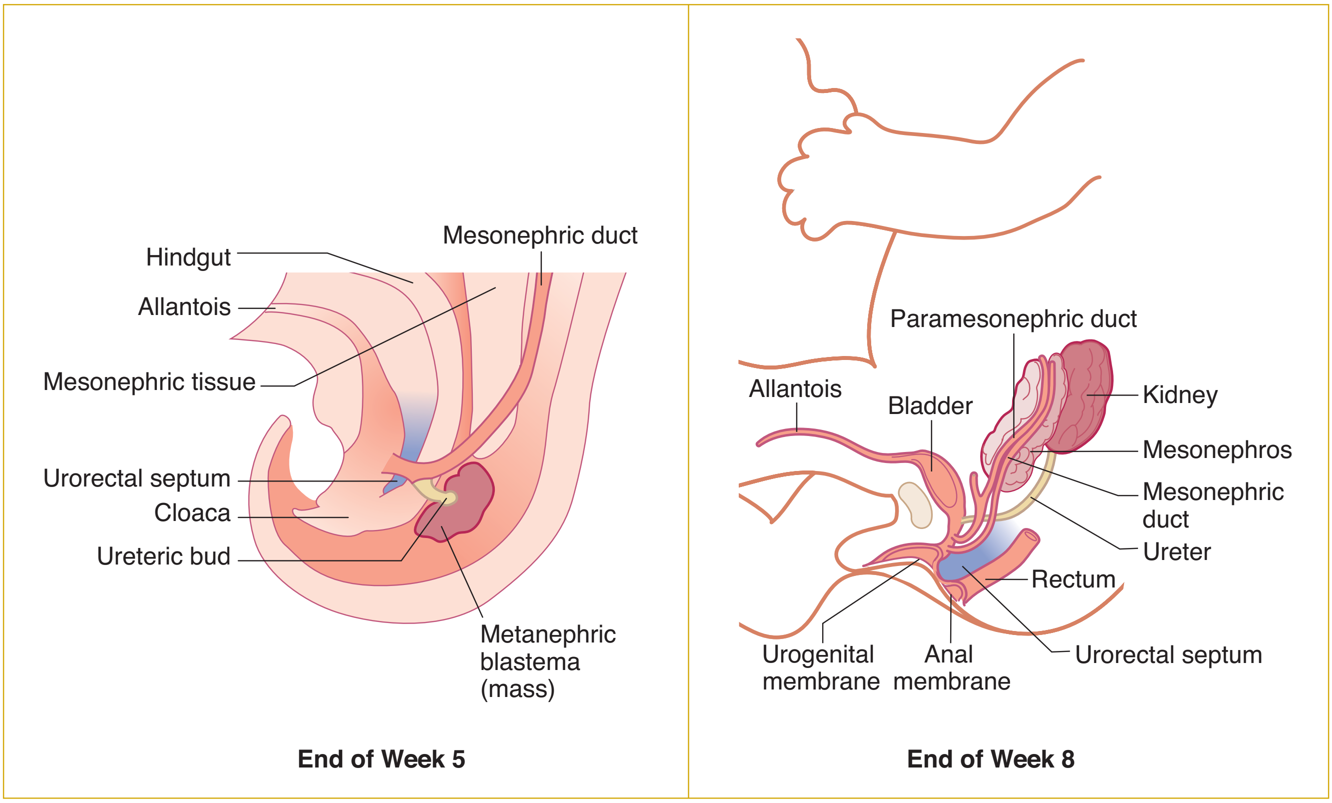

Figure II-3-37. Development of the Urinary System

Embryology of Bladder and Urethra

The **hindgut** does not rotate but it is divided into 2 parts by the **urorectal septum**. The urorectal septum divides the cloaca into the **anorectal canal** and the **urogenital sinus** by week 7.

The **urogenital sinus** is divided into 3 parts:

- The **upper** (cranial) and largest part of the urogenital sinus (endoderm) becomes the **urinary bladder**, which is initially continuous with the **allantois**.
  - The lumen of the allantois becomes obliterated to form a fibrous cord, the **urachus**. The urachus connects the apex of the bladder to the umbilicus. In the adult, this structure becomes the **median umbilical ligament**.
  - The **trigone of the bladder** is formed by the incorporation of the caudal mesonephric ducts into the dorsal bladder wall. This mesodermal tissue is eventually covered by endodermal epithelium so that the entire lining of the bladder is of **endodermal origin**. The smooth muscle of the bladder is derived from splanchnic mesoderm.
  - The mesonephric ducts also form the **ejaculatory ducts** as they enter the prostatic urethra.
- The **middle** part of the urogenital sinus (endoderm) will form all of the **urethra** of the female and the **prostatic, membranous, and proximal spongy urethra** in the male.
  - The **prostate gland** in the male is formed by an endodermal outgrowth of the prostatic urethra.
- The **inferior** part of the sinus forms the **lower vagina** and contributes to the primordia of the penis or the clitoris.

The **anorectal canal** forms the hindgut distally to the pectinate line.

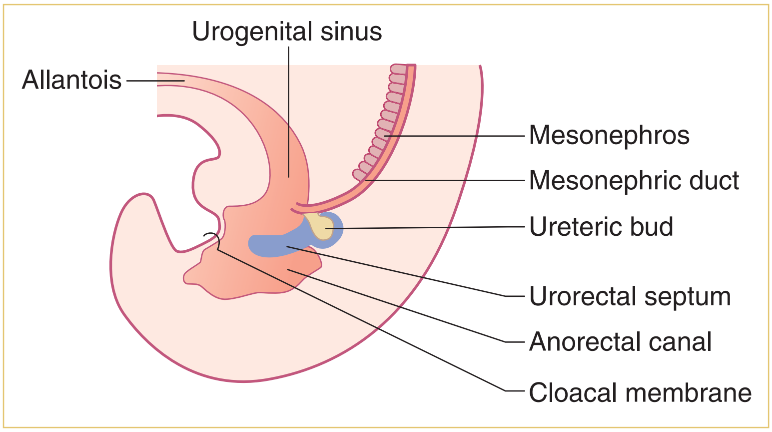

Figure II-3-38. Development of Bladder and Urethra

Congenital Abnormalities of the Renal System

Renal agenesis results from failure of one or both kidneys to develop because of early degeneration of the ureteric bud. Unilateral agenesis is fairly common; bilateral agenesis is fatal (associated with oligohydramnios, and the fetus may have **Potter sequence**: clubbed feet, pulmonary hypoplasia, and craniofacial anomalies).

Pelvic kidney is caused by a failure of one kidney to ascend. **Horseshoe kidney** (usually **normal renal function**, predisposition to calculi) is a fusion of both kidneys at their ends and failure of the fused kidney to ascend. The horseshoe kidney hooks under the origin of the **inferior mesenteric artery**.

Double ureter is caused by the early splitting of the ureteric bud or the development of 2 separate buds.

Failure of the allantois to be obliterated results in **urachal fistulas** or **sinuses**. In male children with congenital valvular obstruction of the prostatic urethra or in older men with enlarged prostates, a **patent urachus may cause drainage of urine through the umbilicus**.

Posterior Abdominal Wall and Pelvic Viscera

The **kidneys** are a pair of bean-shaped organs approximately 12 cm long. They extend from vertebral level T12 to L3 when the body is in the erect position. The right kidney is positioned slightly lower than the left because of the mass of the liver. Both kidneys are in contact with the diaphragm, psoas major, and quadratus lumborum.

- Right kidney: contacts the above structures and the 12th rib
- Left kidney: contacts the above structures and the 11th and 12th ribs

Ureters are fibromuscular tubes that connect the kidneys to the urinary bladder in the pelvis. They run posterior to the ductus deferens in males and posterior to the uterine artery in females. They begin as continuations of the renal pelves and run retroperitoneally, crossing the external iliac arteries as they pass over the pelvic brim. The ureter lies on the **anterior surface** of the **psoas major muscle**.

Clinical Correlate

The most common sites of ureteral constriction susceptible to **blockage by renal calculi** are:

- Where the renal pelvis joins the ureter
- Where the ureter crosses the pelvic inlet
- Where the ureter enters the wall of the urinary bladder

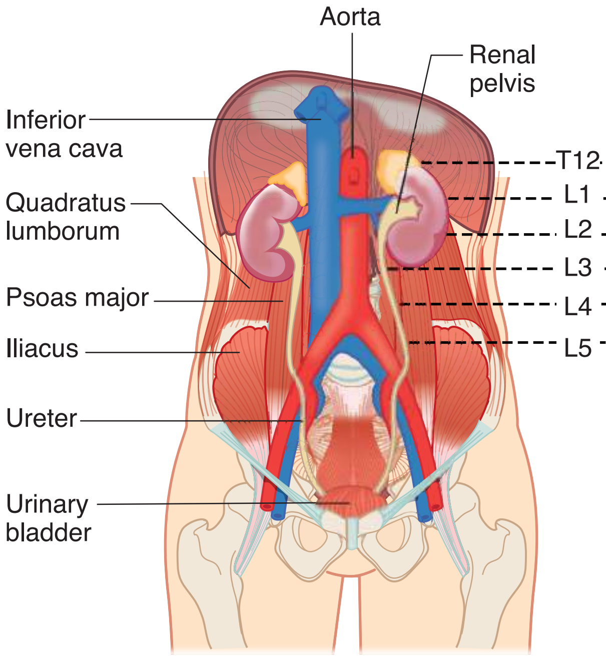

Figure II-3-39A. Muscles of the Posterior Abdominal Wall

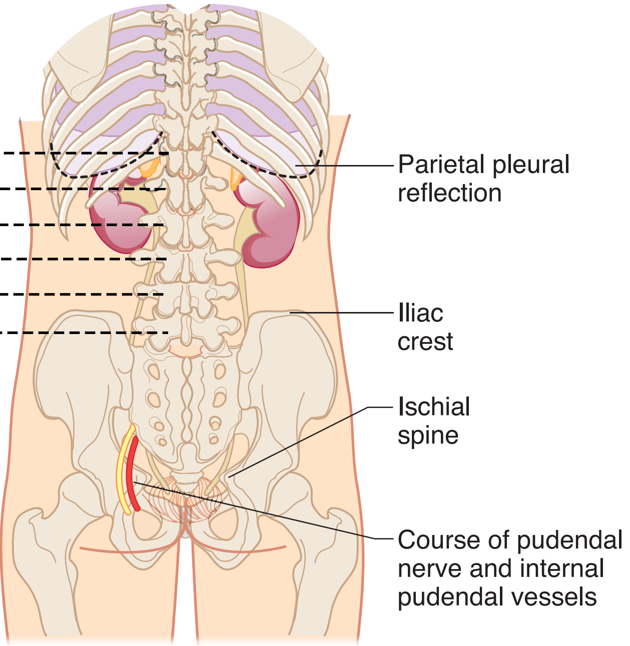

Figure II-3-39B. Bony Landmarks of the Posterior Abdominal Wall

The **urinary bladder** is covered superiorly by peritoneum. The body is a hollow muscular cavity.

- The neck is continuous with the urethra.
- The trigone is a smooth, triangular area of mucosa located internally at the base of the bladder.
- The base of the triangle is superior and bounded by the 2 openings of the ureters. The apex of the trigone points inferiorly and is the opening for the urethra.
- **Blood Supply**
  - The bladder is supplied by vesicular branches of the internal iliac arteries and umbilical arteries.
  - The vesicular venous plexus drains to internal iliac veins.
- **Lymphatics**
  - Drain to the external and internal iliac nodes
- **Innervation**
  - Parasympathetic innervation is from sacral segments S2, S3, and S4. The preganglionic parasympathetic fibers travel in **pelvic splanchnic nerves** to reach the **detrusor muscle**.
  - Sympathetic innervation is through fibers derived from L1 through L2 (**lumbar splanchnics**). These fibers supply the **trigone muscle** and the **internal urethral sphincter**.
- **Muscles**
  - The **detrusor muscle** forms most of the smooth-muscle walls of the bladder and contracts during emptying of the bladder (micturition). The contraction of these muscles is under control of the parasympathetic fibers of the **pelvic splanchnics** (S2, 3, 4)
  - The **internal urethral sphincter** (sphincter vesicae) is smooth-muscle fibers that enclose the origin of the urethra at the **neck of the bladder**. These muscles are under control of the sympathetic fibers of the lower thoracic and **lumbar splanchnics** (T11-L2) and are activated during the filling phase of the bladder to prevent urinary leakage.
  - The **external urethral sphincter** (sphincter urethrae) is the voluntary skeletal muscle component of the urogenital diaphragm that encloses the urethra and is **relaxed** during micturition (voluntary muscle of micturition). The external sphincter is innervated by perineal branches of the **pudendal nerve**.

The **male urethra** is a muscular tube approximately 20 cm in length. The urethra in men extends from the neck of the bladder through the prostate gland (prostatic urethra) to the urogenital diaphragm of the perineum (membranous urethra), and then to the external opening of the glans (penile or spongy urethra).

Note

Parasympathetic fibers facilitate micturition and sympathetic fibers inhibit micturition.

Clinical Correlate

Spastic bladder results from lesions of the spinal cord **above the sacral spinal cord levels**. There is a loss of inhibition of the parasympathetic nerve fibers that innervate the detrusor muscle during the filling stage. Thus, the detrusor muscle responds to a minimum amount of stretch, causing urge incontinence.

Clinical Correlate

Atonic bladder results from lesions **to the sacral spinal cord segments** or the sacral spinal nerve roots. Loss of pelvic splanchnic motor innervation with loss of contraction of the detrusor muscle results in a full bladder with a continuous dribble of urine from the bladder.

Clinical Correlate

Weakness of the **puborectalis** part of the levator ani muscle may result in **rectal incontinence**.

Weakness of the **sphincter urethrae** part of the urogenital diaphragm may result in **urinary incontinence**.

The male urethra is anatomically divided into 3 portions: prostatic, membranous, and spongy (penile). The **distal spongy urethra** of the male is derived from the ectodermal cells of the glans penis.

The **female urethra** is approximately 4 cm in length and extends from the neck of the bladder to the external urethral orifice of the vulva.

URINARY HISTOLOGY AND FUNCTION

The urinary system consists of 2 kidneys, 2 ureters, the bladder, and the urethra.

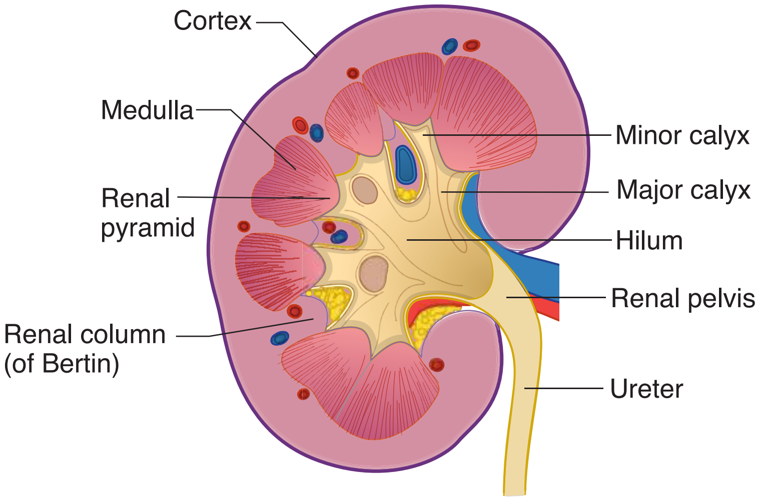

Figure II-3-40. Organization of the Kidney

Urinary Functions

The urinary system functions in the removal of waste products from blood. The kidney also functions in fluid balance, salt balance, and acid-base balance. The kidney functions as an endocrine gland; it produces and releases renin, which leads to an increase in extracellular fluid volume; erythropoietin, which stimulates erythropoiesis; and prostaglandins, which act as vasodilators.

A sagittal section through the center of a kidney shows a capsule (connective tissue) surrounding and protecting the organ, a wide band of cortex showing radial striations and the presence of glomeruli, and a medulla in the shape of an inverted pyramid. The medulla in turn shows an outer and inner zone. The blunted tip of the pyramid, called the **papilla**, borders a space that is surrounded by calices of the ureter. The collecting ducts are invaginations of the papilla's epithelium and the urine drains from their open ends into the calices.

Table II-3-7. Basic Functions of the Kidneys

Fluid balance	Maintain normal extracellular fluid (ECF) and intracellular fluid (ICF) volumes
Electrolytes	Balance excretion with intake to maintain normal plasma concentrations
Wastes	Excrete metabolic wastes (nitrogenous products, acids, toxins, etc.)
Fuels	Reabsorb metabolic fuels (glucose, lactate, amino acids, etc.)
Blood pressure	Regulate ECF volume for the long-term control of blood pressure
Acid–base	Regulate absorption and excretion of H^+ and HCO_3^- to control acid–base balance

Organization of the Kidney

The cortex is divided into lobules, and contains nephron elements mixed with vascular elements and stroma (a small amount of connective tissue). At the center of each lobule is a medullary ray, containing tubules that are parallel to each other and oriented radially in the cortex. The tubules in the medullary rays are continuous with those in the medulla. Along the 2 edges of each lobule are glomeruli, located along 1 or 2 rows. Radially oriented arterioles and venules with a large lumen are located at the edges of the lobules.

The medulla is comprised of radially arranged straight tubules which run from cortex to papilla, vascular elements, and stroma (a small amount of connective tissue). The medulla is divided into 2 zones. A wide strip in proximity to the cortex, the outer medulla contains profiles of tubules with different appearances. The inner medulla has fewer profiles of similar tubes.

Blood Circulation

The renal artery enters the kidney at the hilum, near the ureter. The artery branches into interlobar arteries, which travel to the medulla–cortex border remaining outside the medullary pyramids. The vessels branch into arcuate arteries (and veins) that follow the edge of the cortex. The arcuate arteries branch into interlobular arterioles that travel tangentially in the cortex at the edges of the lobules. Intralobular arterioles, feeding the glomeruli, branch off the interlobular arterioles at each renal corpuscle.

The kidneys receive 25% of total cardiac output, 1,700 liters in 24 hours. Each intralobular arteriole enters a renal corpuscle at the vascular pole as afferent arteriole and forms a convoluted tuft of capillaries (the glomerulus). A second arteriole (the efferent arteriole) exits the corpuscle. This is a unique situation due to the fact that the pressure remains high in the glomerulus in order to allow filtration.

The efferent arterioles carrying blood out of the glomeruli make a second capillary bed. This second capillary bed has lower blood pressure than the glomerulus and it connects to venules at its distal end. The arteriole-capillary-arteriole-capillary-vein sequence in the kidney is unique in the body. The efferent arterioles from glomeruli in the upper cortex divide into a complex capillary system in the cortex.

NEPHRON

The functional unit within the kidney is the nephron. Each kidney contains 1–1.3 million nephrons. Nephrons connect to collecting ducts, and collecting ducts receive urine from several nephrons and converge with each other before opening to and letting the urine flow out of the kidney. The nephron and the collecting duct form the uriniferous tubule.

The nephron is a tube about 55 mm in length in the human kidney. It starts at one end with Bowman's capsule, which is the enlarged end of the nephron. Bowman's capsule has been invaginated by a tuft of capillaries of the glomerulus so that it has 2 layers: the visceral layer is in direct contact with the capillary endothelium, and the parietal layer surrounds an approximately spherical urinary space. Bowman's capsule and glomerulus of capillaries form a renal corpuscle.

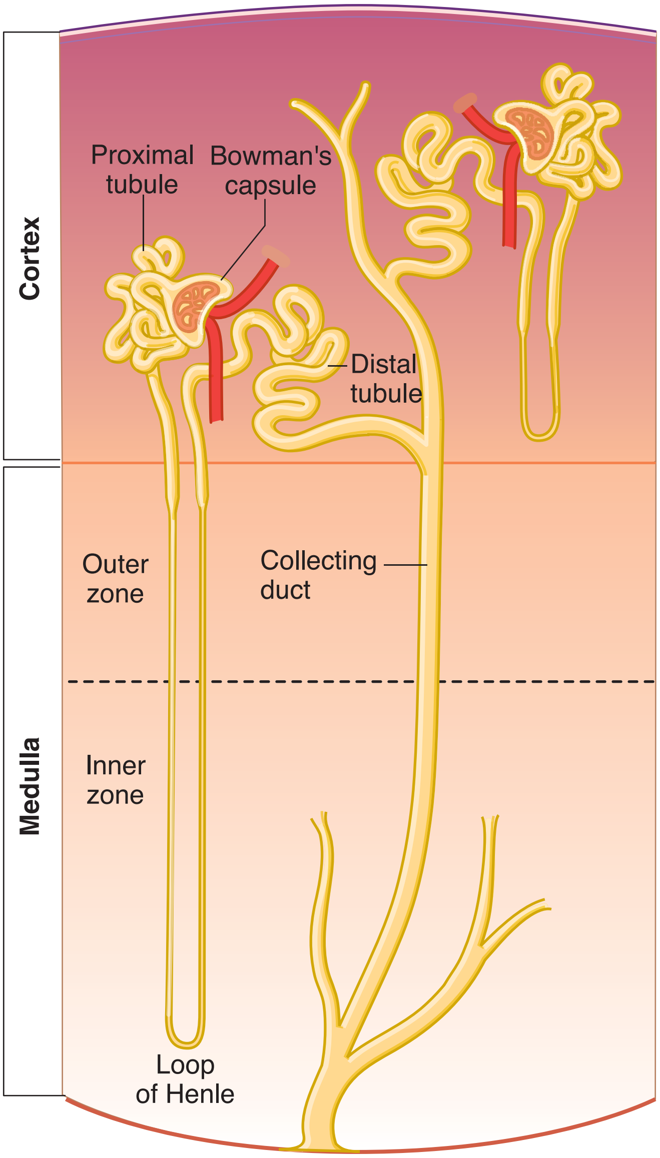

Figure II-3-41. Nephron

Renal Corpuscle

The parietal layer of Bowman's capsule is continuous with the walls of the proximal convoluted tubule (PCT). The visceral layer cells are called podocytes and have a complex shape; the cell body has extensive primary and secondary foot processes which surround the blood vessels. The foot processes of the podocytes lie a basal lamina that is shared by capillary endothelial cells. The podocyte foot processes almost completely cover the capillary surfaces, leaving small slits in between.

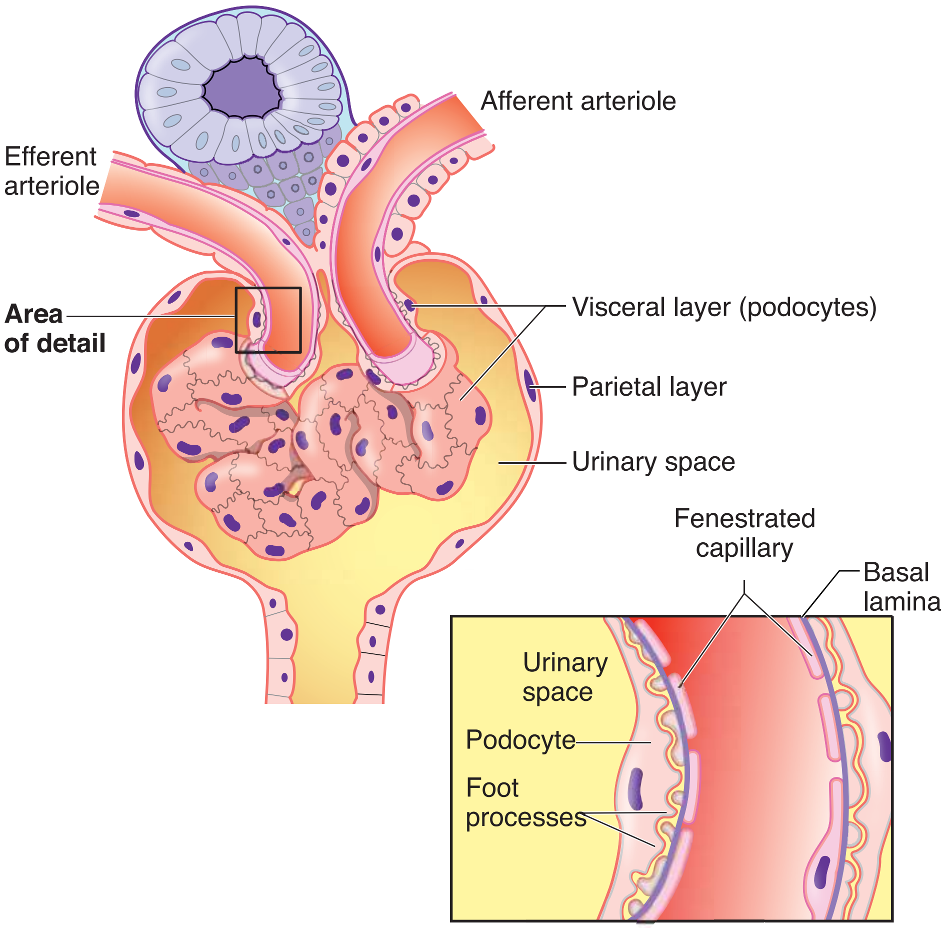

Figure II-3-42. Renal Corpuscle and Bowman's Capsule

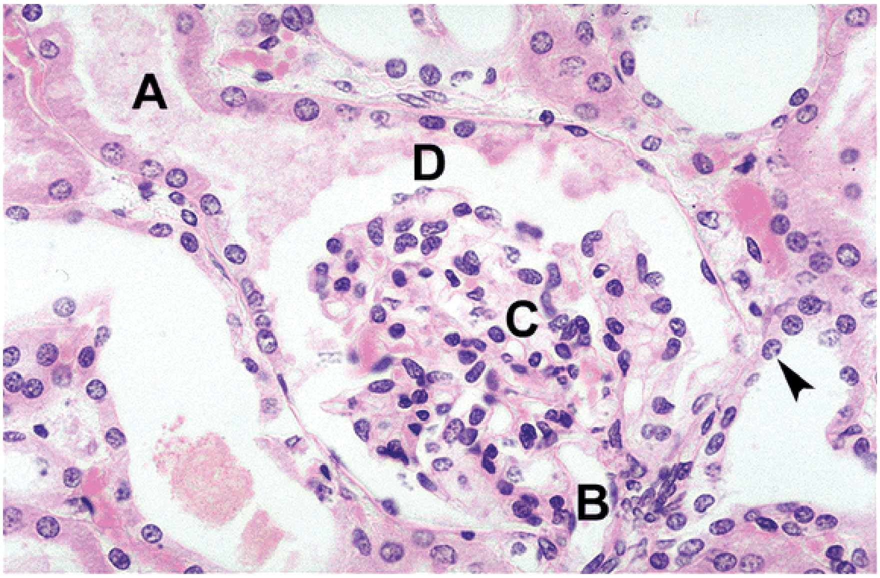

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Figure II-3-43. Renal corpuscle proximal tubule (A), vascular pole (B), glomerulus (C), and urinary space (D)

Simple cuboidal epithelium of the distal tubule (arrowhead)

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Figure II-3-44. Scanning electron micrograph demonstrating podocytes with their processes (arrows)

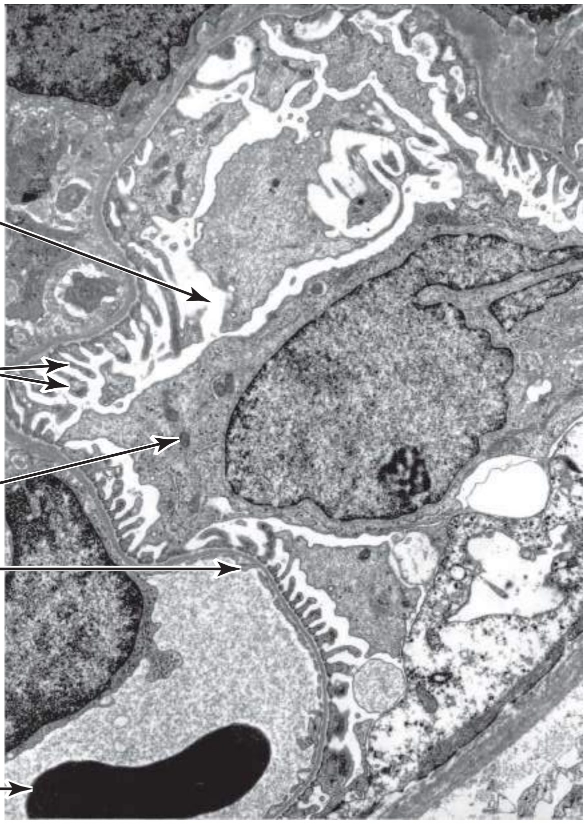

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Figure II-3-45. Transmission electron micrograph demonstrating podocytes

Blood plasma is filtered from the lumen of the capillary to the urinary space across the combined capillary endothelium-podocyte complex. Fenestrations in the endothelium are large (50–100 nm) and occupy 20% of the capillary surface. Fenestrations block the exit of cells, but allow free flow of plasma. The shared basal lamina of podocytes and endothelium constitutes the first, coarser filtration barrier; it blocks the passage of molecules larger than 70 kD.

The thin diaphragms covering the slit openings between the podocyte foot processes constitute a more selective filter. The slits are composed of elongated proteins which arise from the surface of the adjacent foot process cell membranes and join in the center of the slit, in a zipper-like configuration. The width of the junction between 2 adjacent podocytes varies between 20 and 50 nm, possibly as a function of perfusion pressures of the glomerulus.

Podocyte foot processes are motile (they contain actin and myosin). They are connected to each other by the slit diaphragm and to the basal lamina. The slit diaphragm molecular complex is associated with the actin cytoskeleton. Alterations in composition and/or arrangement of these complexes are found in many forms of human and experimental diseases.

Proximal Convoluted Tubule

The proximal convoluted tubule (PCT) opens at the urinary pole of Bowman's capsule. The PCT follows a circuitous path and ends with a straight segment that connects to the loop of Henle. PCT cells are tall, and they have a pink cytoplasm, long apical microvilli, and extensive basal invaginations. Numerous large mitochondria are located between the basal invaginations. The lateral borders of adjacent cells are extensively interdigitated. These characteristics are typical of cells involved in active transport. The lumen of the PCT is frequently clouded by microvilli which do not preserve well during the histologic preparation process.

Loop of Henle

The loop of Henle has a smaller diameter than the PCT and has descending and ascending limbs which go in opposite directions. Some loops of Henle have a wider segment before the distal tubule. The straight and convoluted segments of the distal convoluted tubule (DCT) follow. The straight portions of the PCT and DCT have traditionally been assigned to the loop of Henle (constituting the thick ascending and descending limbs) but they are now thought to be part of the PCT and DCT to which they are more similar. The special disposition of the loops of Henle descending and ascending branches, coupled with their specific transport and permeability properties, allow them to operate as "countercurrent multipliers," creating a gradient of extracellular fluid tonicity in the medulla. This is used to modulate urine tonicity and final volume.

Distal Convoluted Tubule

The DCT comes back to make contact with its own glomerulus, and then connects to the collecting tubule, which receives urine from several nephrons and is open at its far end. The epithelium of DCT, loops of Henle, and collecting ducts have variable thicknesses and more or less well-defined cell borders. Some have limited surface microvilli. In general, these tubes either do much less active transport than the PCT or are involved only in passive water movements.

Note

Renal cortex and medullary fibroblasts (interstitial cells) produce erythropoietin.

Note

Diuretics act by inhibiting Na^+ resorption, leading to an increase in Na^+ and water excretion.

Collecting Ducts

Collecting ducts are lined by principal cells and intercalated cells. The cell outline of these cells is more distinct than that of the PCT or the DCT. Principal cells respond to aldosterone.

Mesangial Cells

Mesangial cells (also known as Polkissen or Lacis cells) are located between capillaries, under the basal lamina but outside the capillary lumen. There is no basal lamina between mesangial and endothelial cells. Mesangial cells are phagocytic and may be involved in the maintenance of the basal lamina. Abnormalities of mesangial cells are detected in several diseases resulting in clogged and/or distorted glomeruli.

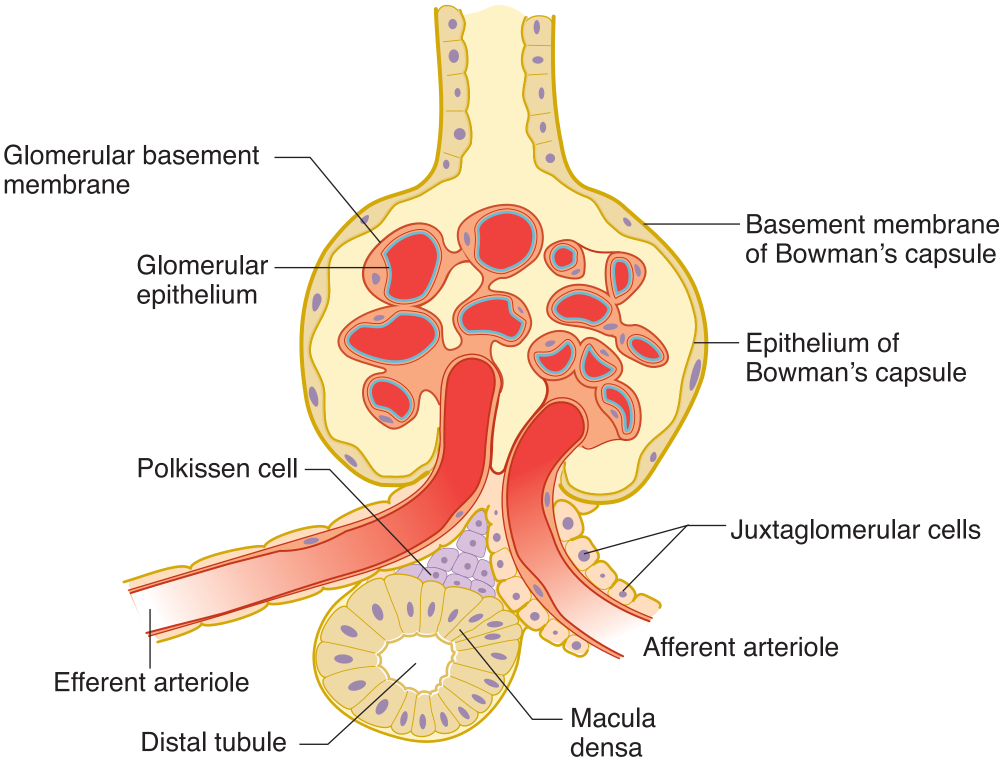


Figure II-3-46. Renal Corpuscle and Juxtaglomerular Apparatus

Juxtaglomerular Complex

The juxtaglomerular (JG) complex is a complex comprising JG apparatus (in the wall of the afferent arteriole), the macula densa (a special domain of the DCT), and a group of mesangial cells. The JG cells are modified smooth-muscle cells which secrete renin.

The macula densa is formed by tall cuboidal cells in the wall of the DCT which detect sodium levels in the tubular fluid.

PELVIS

Embryology of the Reproductive System

Table II-3-8. Embryology of Reproductive System

Male and Female Development			
Adult Female and Male Reproductive Structures Derived From Precursors of the Indifferent Embryo			
Adult Female	**Indifferent Embryo**	**Adult Male**	
Ovary, follicles, rete ovarii	Gonads **TDF** —+→	Testes, seminiferous tubules, rete testes	
Uterine tubes, uterus, cervix, and upper part of vagina	← Paramesonephric ducts **MIF** —−→	Appendix of testes	
Duct of Gartner	Mesonephric ducts **Testosterone** —+→	Epididymis, ductus deferens, seminal vesicle, ejaculatory duct	
Clitoris	Genital tubercle	Glans and body of penis	**DHT**
Labia minora	Urogenital folds	Ventral aspect of penis	**DHT**
Labia majora	Labioscrotal swellings	Scrotum	**DHT**

Abbreviations: DHT, dihydrotestosterone; MIF, Müllerian-inhibiting factors; TDF, testes-determining factor

Congenital Reproductive Anomalies

Female Pseudointersexuality

- 46,XX genotype
- Have ovarian (but no testicular) tissue and masculinization of the female external genitalia
- Most common cause is **congenital adrenal hyperplasia**, a condition in which the fetus produces excess androgens

Male Pseudointersexuality

- 46,XY genotype
- Testicular (but no ovarian) tissue and stunted development of male external genitalia
- Most common cause is inadequate production of dihydrotestosterone due to a 5α-reductase deficiency

5α-reductase 2 deficiency

- Caused by a mutation in the **5α-reductase 2 gene** that renders 5α-reductase 2 enzyme underactive in catalyzing the conversion of testosterone to dihydrotestosterone
- *Clinical findings:* underdevelopment of the penis and emission of sperm (microphallus, hypospadias, and bifid scrotum) and prostate. The epididymis, ductus deferens, seminal vesicle, and ejaculatory duct are normal
- At puberty, these patients undergo virilization due to an increased **T: DHT ratio**.

Complete androgen insensitivity (CAIS, or testicular feminization syndrome)

- Occurs when a fetus with a 46,XY genotype develops testes and female external genitalia with a rudimentary vagina; the uterus and uterine tubes are generally absent
- Testes may be found in the labia majora and are surgically removed to circumvent malignant tumor formation.
- Individuals present as normal-appearing females, and their psychosocial orientation is female despite their genotype.
- Most common cause is a mutation in the **androgen receptor (AR) gene** that renders the AR inactive.

Abnormalities of the Penis and Testis

Hypospadias

- Occurs when the urethral folds fail to fuse completely, resulting in the external urethral orifice opening onto the **ventral** surface of the penis.
- Generally associated with a poorly developed penis that curves ventrally, known as **chordee.**

Epispadias

- Occurs when the external urethral orifice opens onto the **dorsal** surface of the penis.
- Generally associated with exstrophy of the bladder.

Undescended testes (cryptorchidism)

Occurs when the testes fail to descend into the scrotum. Normally occurs within 3 months after birth.

- Bilateral cryptorchidism results in sterility.
- The undescended testes may be found in the abdominal cavity or in the inguinal canal.

Hydrocele of the testes

Occurs when a small patency of the processus vaginalis remains so that peritoneal fluid can flow into the processus vaginalis. Results in a fluid-filled cyst near the testes.

Pelvic and Urogenital Diaphragms

The **pelvic and urogenital diaphragms** are 2 important skeletal muscle diaphragms that provide support of the pelvic and perineal structures. They are each innervated by branches of the pudendal nerve.

- The **pelvic diaphragm** forms the muscular floor of the pelvis and separates the pelvic cavity from the perineum. The pelvic diaphragm is a strong support for the pelvic organs and transmits the distal parts of the genitourinary system and GI tract from the pelvis to the perineum.
  - The diaphragm is formed by 2 layers of fascia and the 2 muscles: the **levator ani** and coccygeus.
  - The **puborectalis** component of the levator ani muscle forms a muscular sling around the anorectal junction, marks the boundary between the rectum and anal canal, and is important in fecal continence.
- The muscular **urogenital diaphragm** is located in the perineum inferior to the pelvic diaphragm. It is formed by 2 muscles (**sphincter urethrae** and deep transverse perineus muscles) which extend horizontally between the 2 ischiopubic rami.
  - The diaphragm is penetrated by the urethra in the male and the urethra and vagina in the female.
  - The sphincter urethrae muscle serves as an **external urethral sphincter** (voluntary muscle of micturition) which surrounds the membranous urethra and maintains urinary continence.

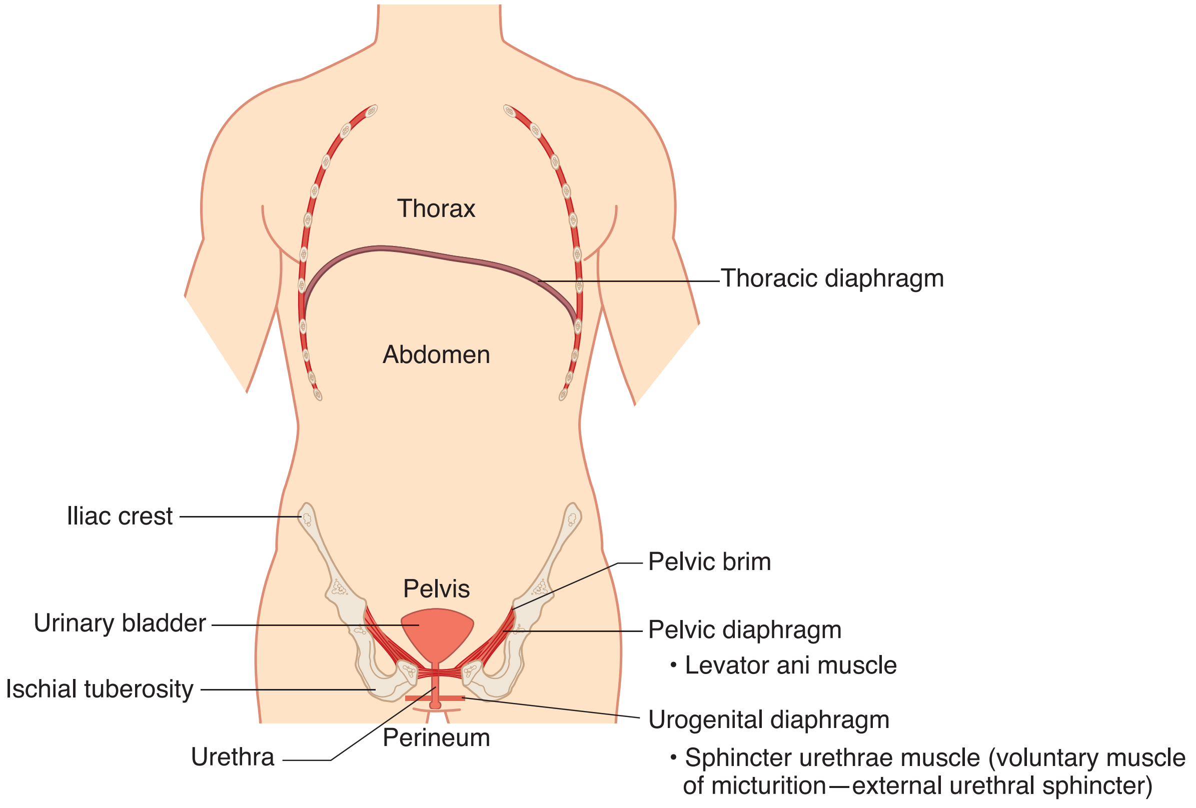


Figure II-3-47. Pelvic Diaphragm

Male Pelvic Viscera

The position of organs and peritoneum in the male pelvis is illustrated below.

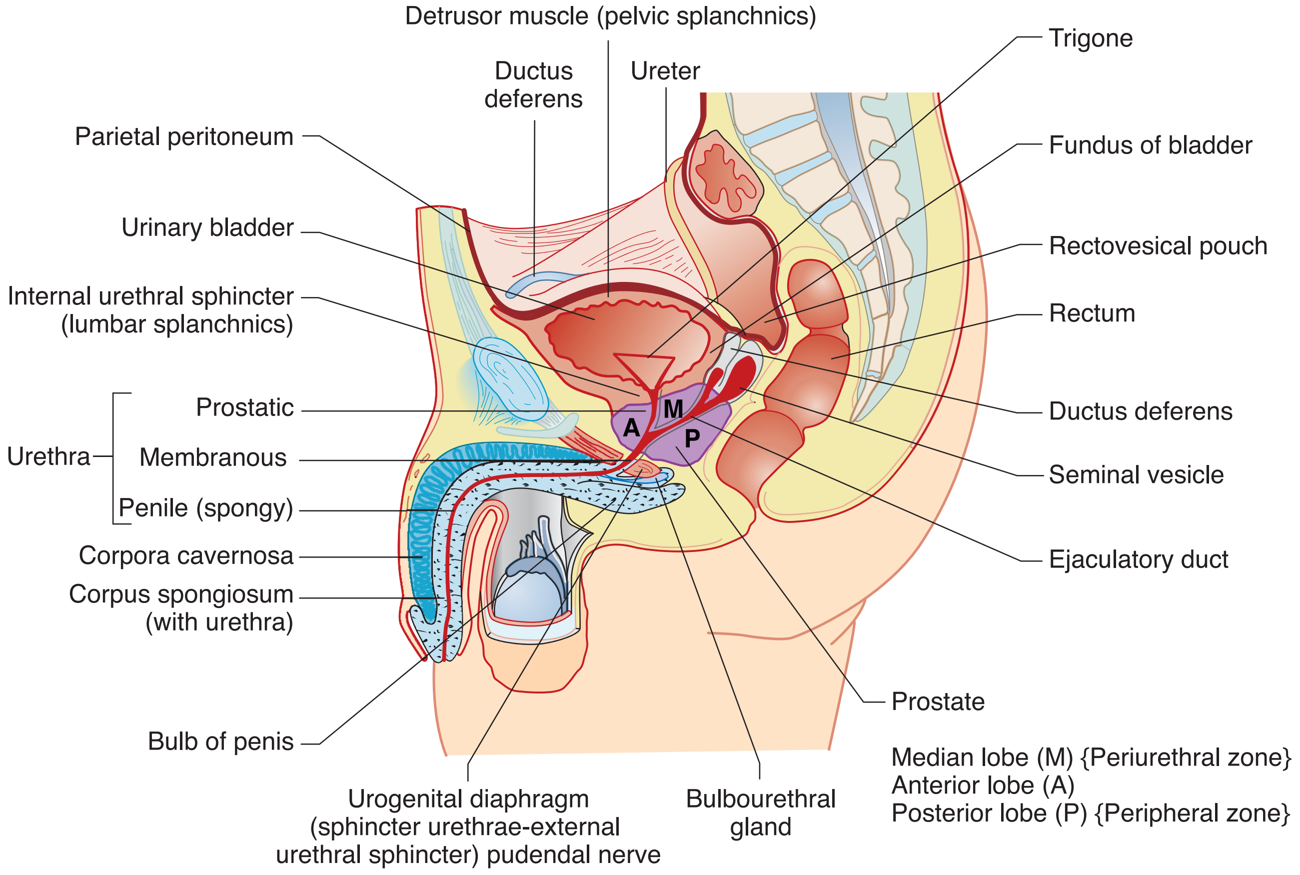


Figure II-3-48. Male Pelvis

Clinical Correlate

Hyperplasia of the Prostate

An enlarged prostate gland will compress the urethra. The patient will complain of the urge to urinate often and has difficulty with starting urination.

Because the prostate gland is enclosed in a dense connective tissue capsule, hypertrophy will compress the prostatic portion of the urethra.

Female Pelvic Viscera

The position of organs and peritoneum in the female pelvis is illustrated below.

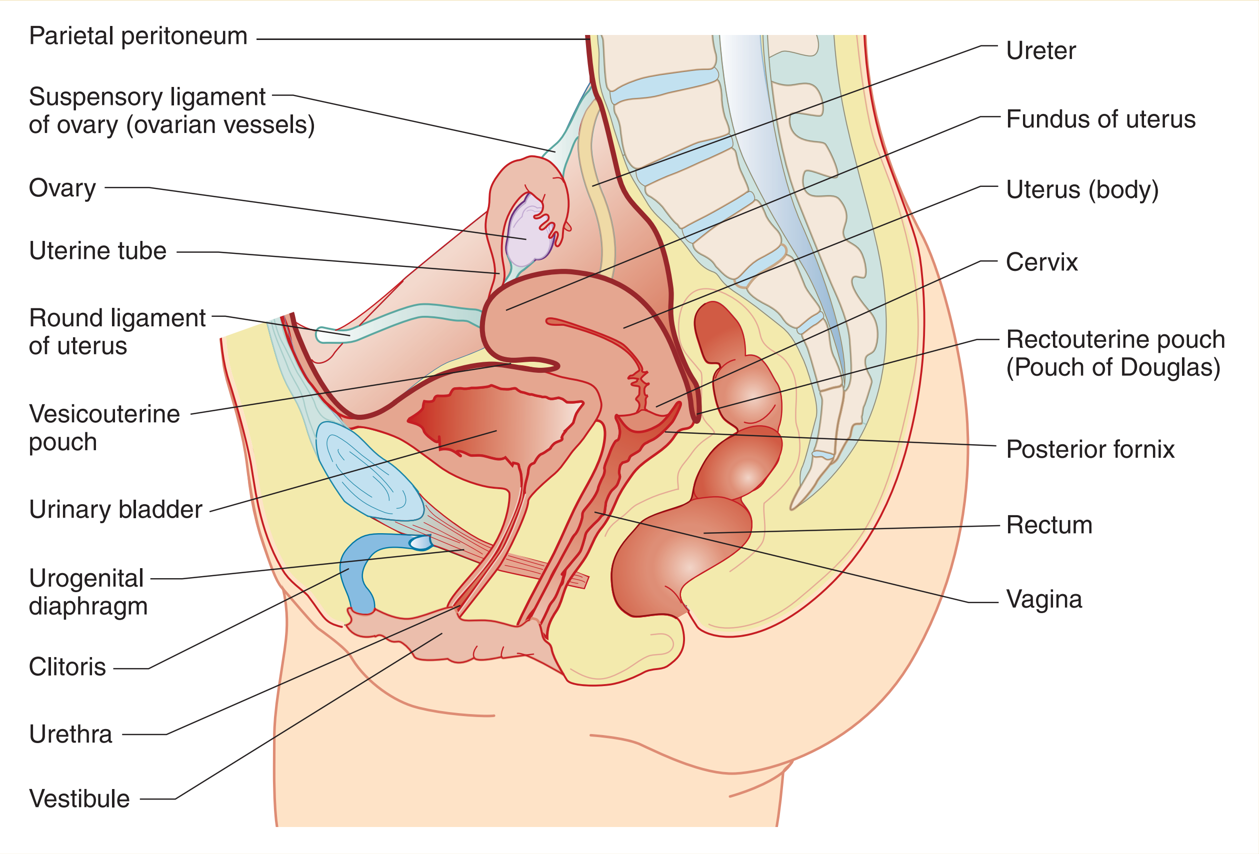


Figure II-3-49. Female Pelvis

Clinical Correlate

The ureter courses just medial to the suspensory ligament of the ovary and must be protected when ligating the ovarian vessels.

Clinical Correlate

Support for pelvic viscera is provided by the pelvic and urogenital diaphragms, perineal membrane, perineal body, and the transverse (cardinal) cervical and uterosacral ligaments. Weakness of support structures may result in **prolapse** of the uterus into the vagina or **herniation** of the bladder or rectum into the vagina.

Clinical Correlate

The ureter passes **inferior** to the uterine artery 1 to 2 centimeters from the cervix ("water under the bridge") and must be avoided during surgical procedures.

Clinical Correlate

A **pudendal nerve block** to anesthetize the perineum is performed as the pudendal nerve crosses posterior to the **ischial spine**.

Uterus and Broad Ligament

A posterior view of the female reproductive tract is shown below.

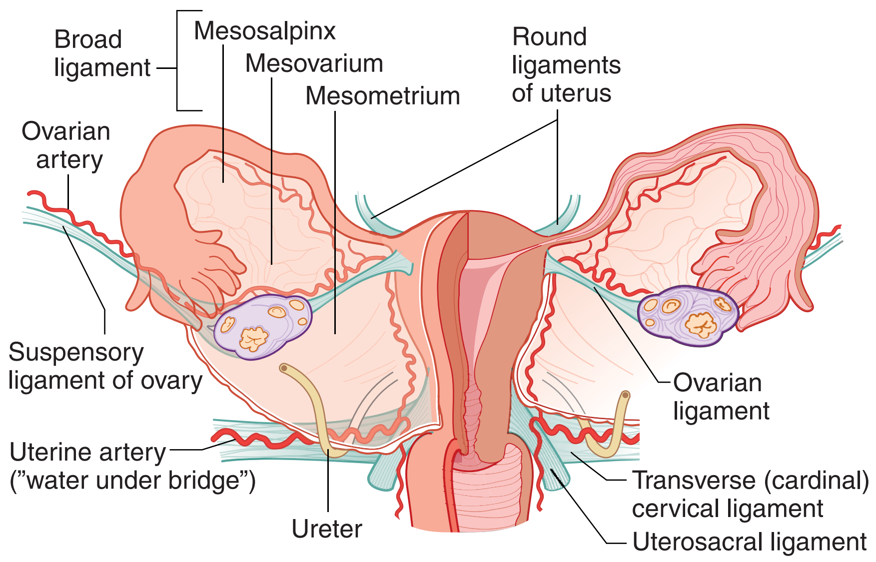


Figure II-3-50. Broad Ligament

PERINEUM

The **perineum** is the diamond-shaped outlet of the pelvis located below the pelvic diaphragm. It is divided by a transverse line between the ischial tuberosities into the **anal** and **urogenital triangles**.

- The sensory and motor innervation to the perineum is provided by the **pudendal nerve** (S2, 3, 4) of the sacral plexus.
- The blood supply is provided by the **internal pudendal artery,** a branch of the internal iliac artery.
- The pudendal nerve and vessels cross the ischial spine posteriorly to enter the perineum.

Anal Triangle

The **anal triangle** is posterior and contains the **anal canal** surrounded by the fat-filled **ischioanal fossa**. The anal canal is guarded by a smooth-muscle **internal anal sphincter** innervated by the ANS and an **external anal sphincter** of skeletal muscle innervated by the pudendal nerve. The pudendal canal transmitting the pudendal nerve and internal pudendal vessels is found on the lateral aspect of the ischioanal fossa.

Urogenital Triangle

The **urogenital triangle** forms the anterior aspect of the perineum and contains the superficial and root structures of the external genitalia. The urogenital triangle is divided into **superficial** and **deep perineal spaces (pouches)**.

The **superficial perineal pouch** is located between the **perineal membrane** of the urogenital diaphragm and the **superficial perineal (Colles') fascia**. It contains:

- Crura of penis or clitoris: erectile tissue
- Bulb of penis (in the male): erectile tissue; contains urethra
- Bulbs of vestibule (in the female): erectile tissue in lateral walls of vestibule
- Ischiocavernosus muscle: skeletal muscle that covers crura of penis or clitoris
- Bulbospongiosus muscle: skeletal muscle that covers bulb of penis or bulb of vestibule
- Greater vestibular (Bartholin) gland (in female only): homologous to Cowper gland

The **deep perineal pouch** is formed by the fasciae and muscles of the **urogenital diaphragm**. It contains:

- Sphincter urethrae muscle—serves as voluntary external sphincter of the urethra
- Deep transverse perineal muscle
- Bulbourethral (Cowper) gland (in the male only)—duct enters bulbar urethra

Note

The **bulbourethral (Cowper) glands** are located in the **deep perineal pouch** of the male.

The **greater vestibular (Bartholin) glands** are located in the **superficial perineal pouch** of the female.

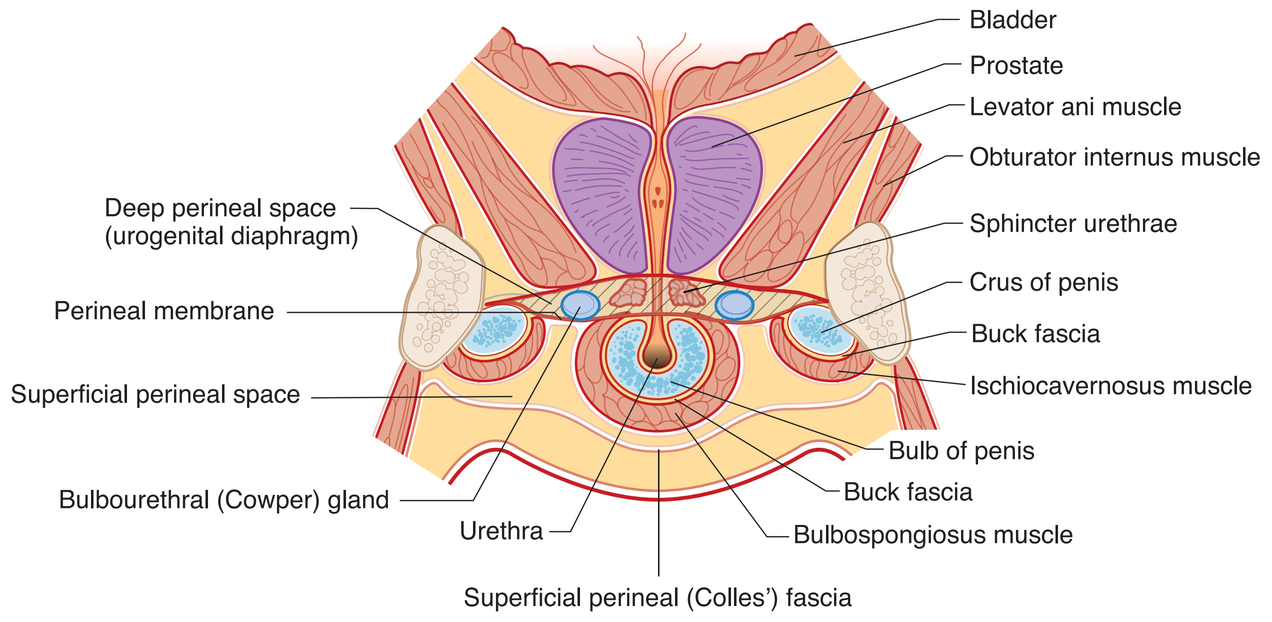


Figure II-3-51. Superficial and Deep Perineal Pouches of Male

External Genitalia

Male

Crura of penis are continuous with the corpora cavernosa of the penis.

Bulb of penis is continuous with corpus spongiosus of the penis (contains urethra).

Corpora cavernosa and corpus spongiosus form the shaft of the penis.

In the male, injury to the bulb of the penis (blue arrow) may result in extravasation of urine from the urethra into the superficial perineal space. From this space, urine may pass into the scrotum, into the penis, and onto the anterior abdominal wall in the plane deep to Scarpa fascia (green arrows).

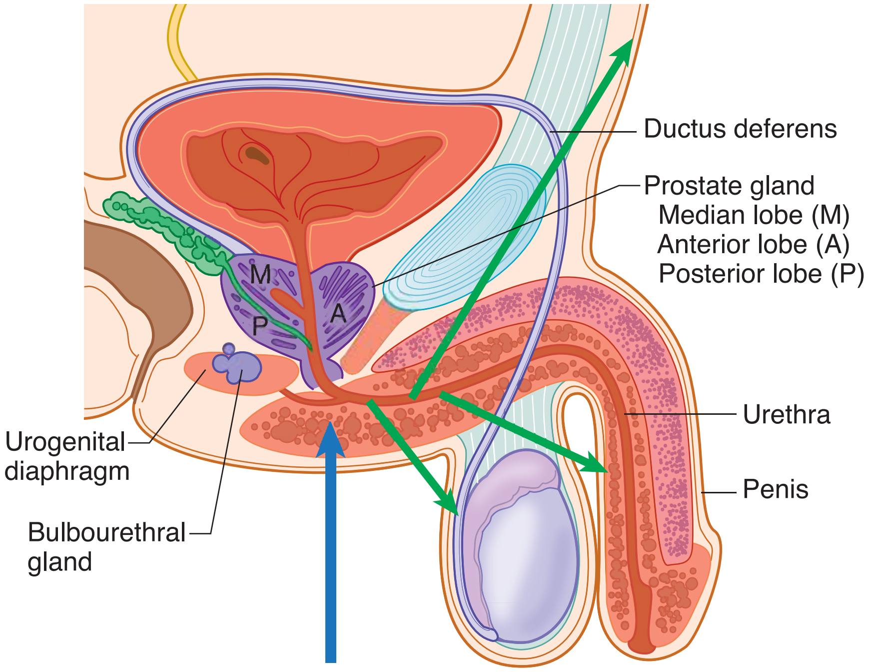


Figure II-3-52. Male Urethra

Female

Crura of the clitoris are continuous with the corpora cavernosa of the clitoris.

Bulbs of vestibule are separated from the vestibule by the labia minora.

Urethra and vagina empty into the vestibule.

Duct of greater vestibular glands enters the vestibule.

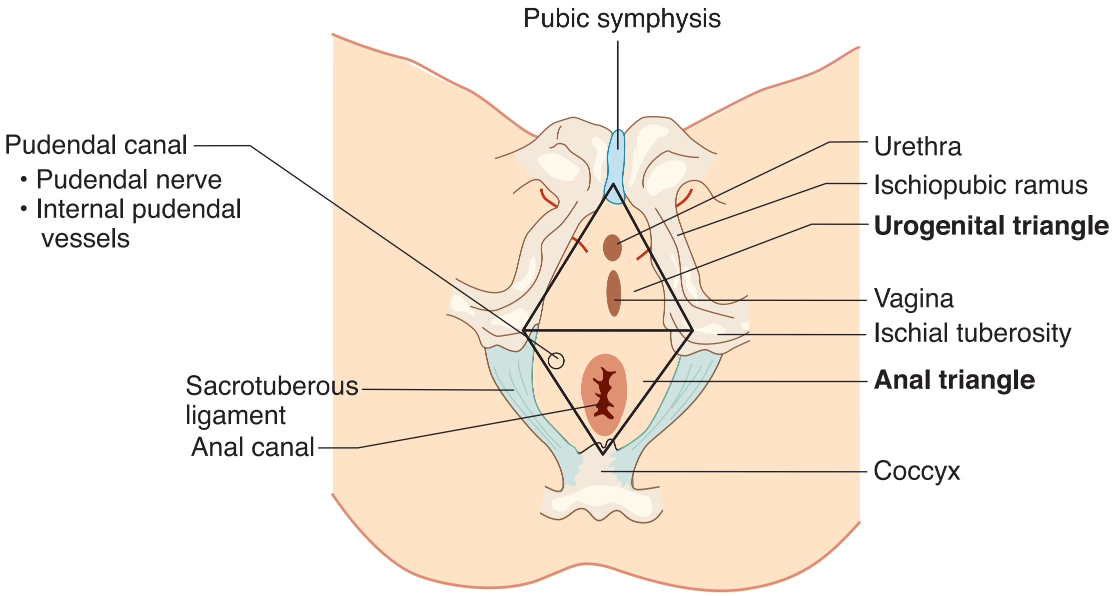


Figure II-3-53. Perineum of Female

Pelvic and Perineal Innervation

The **pudendal nerve (S2, S3, S4 ventral rami) and its branches** innervate the skeletal muscles in the pelvic and urogenital diaphragms, the external anal sphincter and the sphincter urethrae, skeletal muscles in both perineal pouches, and the skin that overlies the perineum.

MALE REPRODUCTIVE HISTOLOGY

Testis

The testis is surrounded by a dense fibrous capsule called the **tunica albuginea**. The tunica albuginea is continuous with many of the interlobular septa that divide the testis into approximately 250 pyramidal compartments (testicular lobules).

Within each lobule are 1-4 tubes, **seminiferous tubules**, where spermatozoa are produced. Each tubule is a coiled, non-branching closed loop that is 150–200 μm in diameter and 30–70 cm in length. Both ends of each tubule converge on the rete testes. The seminiferous tubules contain spermatogenic cells, Sertoli cells, and a well-defined basal lamina.

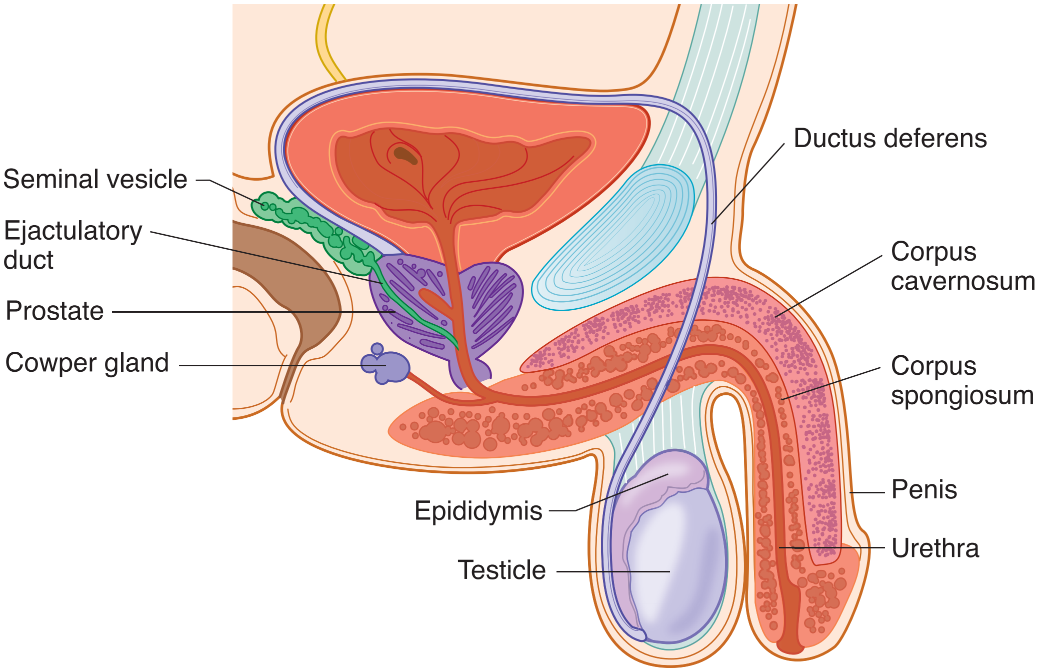
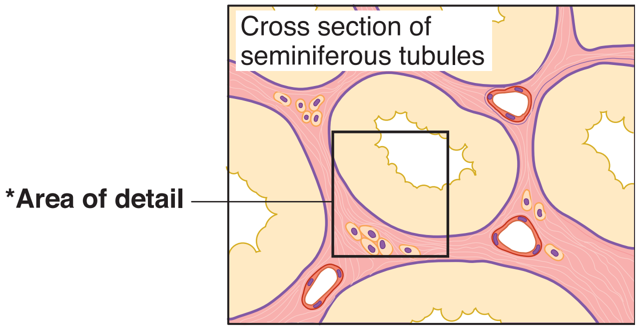
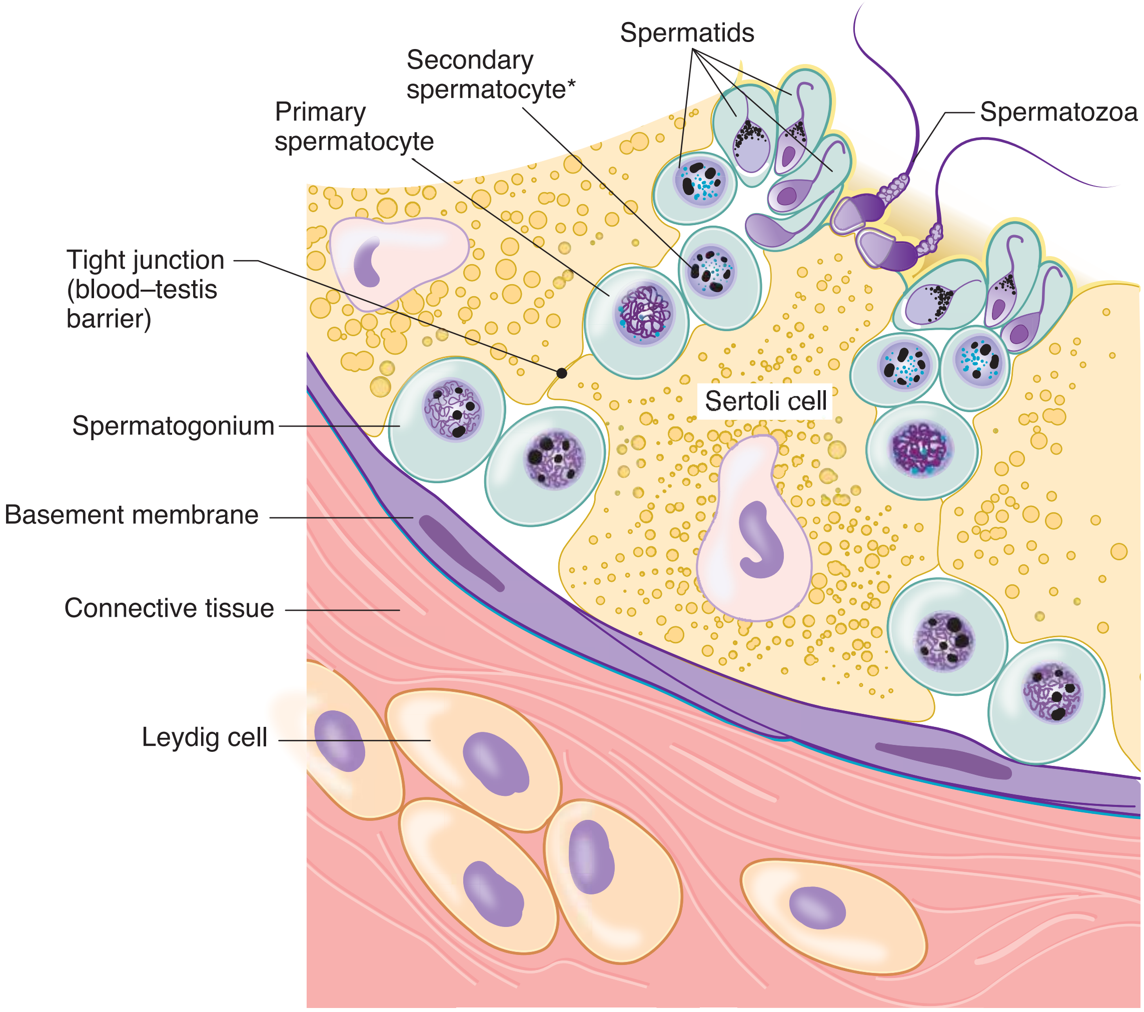


Figure II-3-54. Male Reproductive System

Note

The blood–testis barrier is formed by tight junctions between Sertoli cells and protects primary spermatocytes and their progeny.





*least likely to be seen

Figure II-3-55. Seminiferous Tubule Diagram

Spermatogenesis

The spermatogenic cells (germinal epithelium) are stacked in 4 to 8 layers that occupy the space between the basement membrane and the lumen of the seminiferous tubule. The stem cells (**spermatogonia**) are adjacent to the basement membrane. As the cells develop, they move from the basal to the luminal side of the tubule.

At puberty the stem cells resume mitosis, producing more stem cells as well as differentiated spermatogonia (type A and B) that are committed to meiosis. **Type B spermatogonia** differentiate into primary spermatocytes that enter meiosis. **Primary spermatocytes** (4n, diploid) pass through a long prophase (10 days to 2 weeks) and after the first meiotic division form 2 secondary spermatocytes (2n, haploid). The **secondary spermatocytes** rapidly undergo the second meiotic division in a matter of minutes **(and are rarely seen in histologic sections)** to produce the **spermatids** (1n, haploid).

The progeny of a single maturing spermatogonium remain connected to one another by cytoplasmic bridges throughout their differentiation into mature sperm.

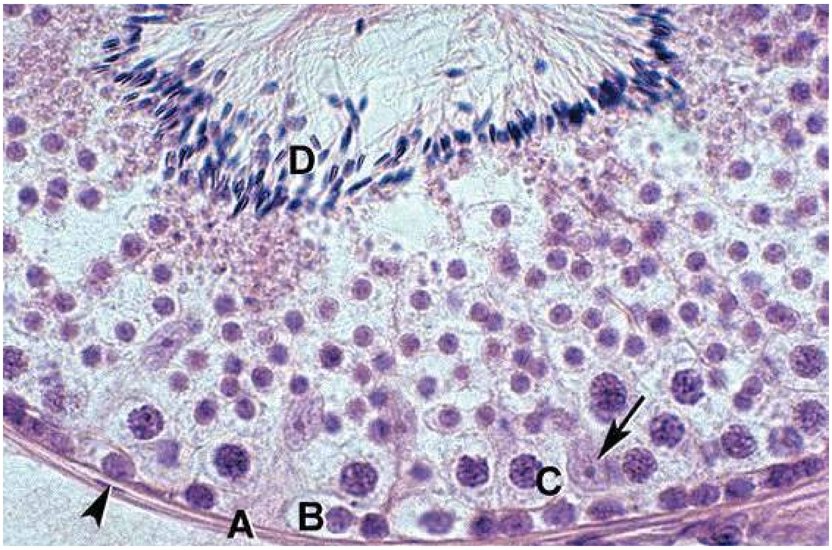


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Figure II-3-56. Seminiferous tubule surrounded by a basement membrane (A) and myoepithelial cells

Spermatogonia (B) lie on the basement membrane, primary spermatocytes (C), and spermatozoa (D) are inside the blood testis barrier. Sertoli cells (arrow) have elongated, pale-staining nuclei.

Spermiogenesis

Spermiogenesis transforms haploid spermatids into spermatozoa. This process of differentiation involves formation of the acrosome, condensation, and elongation of the nucleus; development of the flagellum; and loss of much of the cytoplasm.

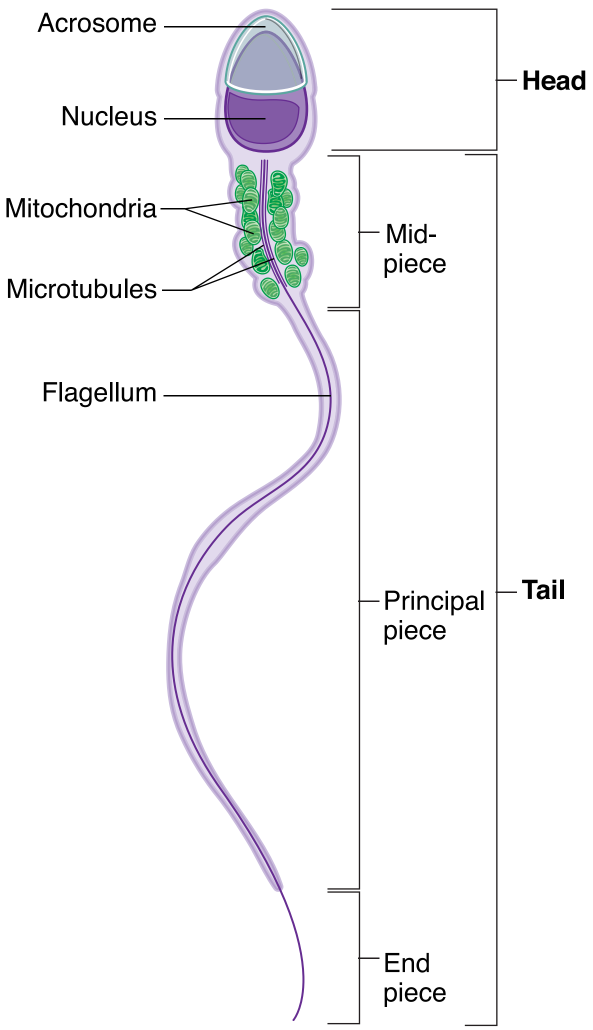

Figure II-3-57. Spermatozoan

The **acrosome**, which is located over the anterior half of the nucleus, is derived from the Golgi complex of the spermatid and contains several hydrolytic enzymes such as hyaluronidase, neuraminidase, and acid phosphatase; these enzymes dissociate cells of the corona radiata and digest the zona pellucida of the recently produced secondary oocyte.

The basic structure of a flagellum is similar to that of a cilium. Movement is a result of the interaction among microtubules, ATP, and dynein.

Sertoli Cells and the Blood–Testis Barrier

Sertoli cells are tall columnar epithelial cells. These multifunctional cells are the predominant cells in the seminiferous tubule prior to puberty and in elderly men but comprise only 10% of the cells during times of maximal spermatogenesis.

- Irregular in shape; the base adheres to the basal lamina and the apical end extends to the lumen. The nucleus tends to be oval with the long axis oriented perpendicular to the basement membrane.
- The cytoplasmic extensions make contact with neighboring Sertoli cells via tight junctions, forming the **blood–testis barrier** by separating the seminiferous tubule into a basal and an adlumenal compartment.
- Do not divide during the reproductive period.
- Support, protect, and provide nutrition to the developing spermatozoa. During spermiogenesis, the excess spermatid cytoplasm is shed as residual bodies that are phagocytized by Sertoli cells. They also phagocytize germ cells that fail to mature.
- Secrete **androgen-binding protein** that binds testosterone and dihydrotestosterone. High concentrations of these hormones are essential for normal germ-cell maturation. The production of androgen-binding protein is stimulated by **follicle-stimulating hormone** (FSH receptors are on Sertoli cells).
- Secrete **inhibin**, which suppresses FSH synthesis.
- Produce anti-Müllerian hormone during fetal life that suppresses the development of female internal reproductive structures.

The blood–testis barrier is a network of Sertoli cells which divides the seminiferous tubule into a basal compartment (containing the spermatogonia and the earliest primary spermatocytes) and an adlumenal compartment (containing the remaining spermatocytes and spermatids). The basal compartment has free access to material found in blood, while the more advanced stages of spermatogenesis are protected from blood-borne products by the **barrier** formed by the tight junctions between the Sertoli cells. The primary spermatocytes traverse this barrier by a mechanism not yet understood.

Interstitial Tissues of the Testis

The interstitial tissue lying between the seminiferous tubules is a loose network of connective tissue composed of fibroblasts, collagen, blood and lymphatic vessels, and **Leydig cells** (also called interstitial cells). The Leydig cells synthesize testosterone.

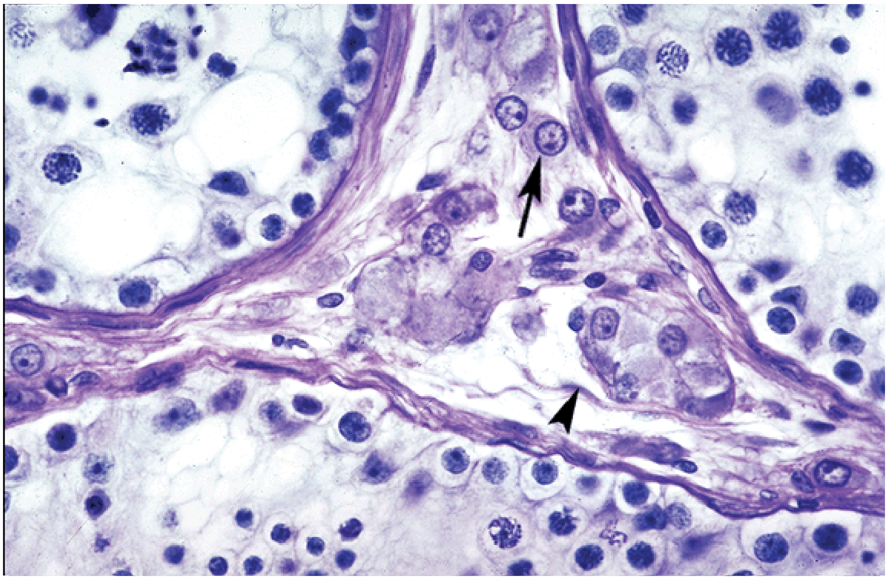

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Figure II-3-58. Interstitium between seminiferous tubules contains Leydig cells (arrow) and fibroblasts (arrowhead)

Genital Ducts

The seminiferous tubules empty into the **rete testis** and then into 10–20 **ductuli efferentes**. The ductuli are lined by a single layer of epithelial cells, some of which are ciliated. The ciliary action propels the nonmotile spermatozoa. The non-ciliated cells reabsorb some of the fluid produced by the testis. A thin band of smooth muscle surrounds each ductus.

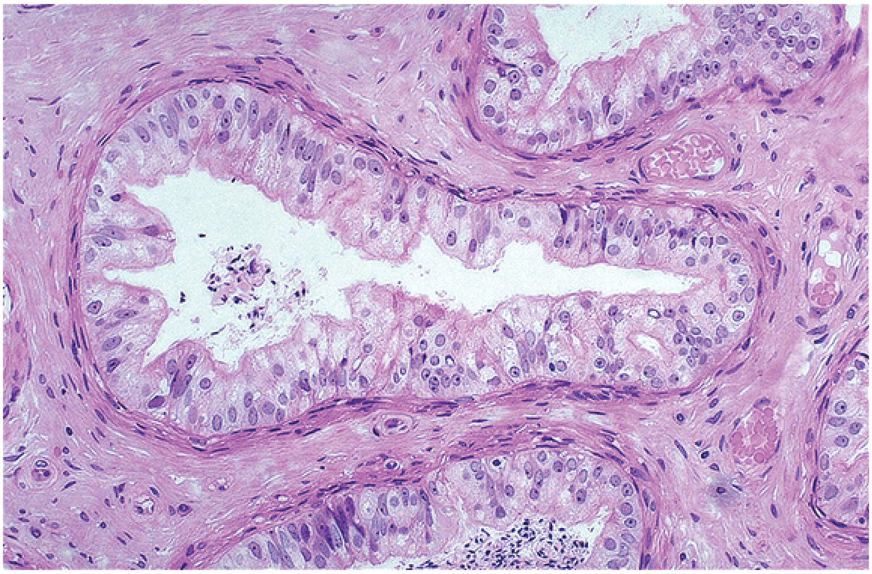

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Figure II-3-59. Efferent ductules with ciliated cuboidal and columnar cells

The spermatozoa pass from the ducti efferentes to the **epididymi**s. The major function of this highly convoluted duct (approximately 5 m long) is the accumulation, storage, and maturation of spermatozoa. It is in the **epididymis** that the spermatozoa become motile. The epididymis is lined with a pseudostratified columnar epithelium which contains stereocilia (tall microvilli) on the luminal surface. This epithelium resorbs testicular fluid, phagocytizes residual bodies and poorly formed spermatozoa, and secretes substances thought to play a role in the maturation of spermatozoa.

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Figure II-3-60. Epididymis lined by pseudostratified columnar epithelium with stereocilia (arrow)

The **ductus (vas) deferens** conducts spermatozoa from the epididymis to the ejaculatory duct and then into the prostatic **urethra**. The ductus (vas) deferens is a thick walled muscular tube consisting of an inner and outer layer of longitudinal smooth muscle and an intermediate circular layer. Vasectomy or the bilateral ligation of the vas deferens prevents movement of spermatozoa from the epididymis to the urethra.

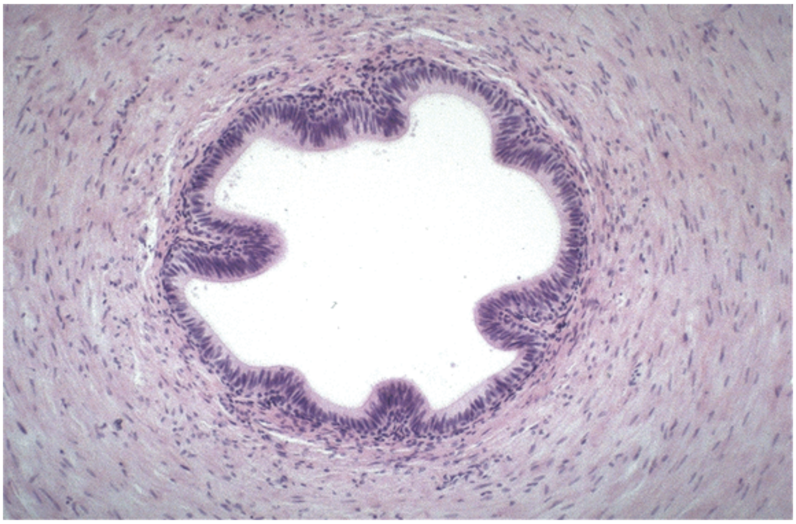

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Figure II-3-61. Ductus deferens with thick layers of smooth muscle

Accessory Glands

The **seminal vesicles** are a pair of glands situated on the posterior and inferior surfaces of the bladder. These highly convoluted glands have a folded mucosa lined with pseudostratified columnar epithelium. The columnar epithelium is rich in secretory granules that displace the nuclei to the cell base.

The seminal vesicles produce a secretion that constitutes approximately 70% of human ejaculate and is rich in spermatozoa-activating substances such as fructose, citrate, prostaglandins, and several proteins. Fructose, which is a major nutrient for sperm, provides the energy for motility. The duct of each seminal vesicle joins a ductus deferens to form an ejaculatory duct. The ejaculatory duct traverses the prostate to empty into the prostatic urethra.

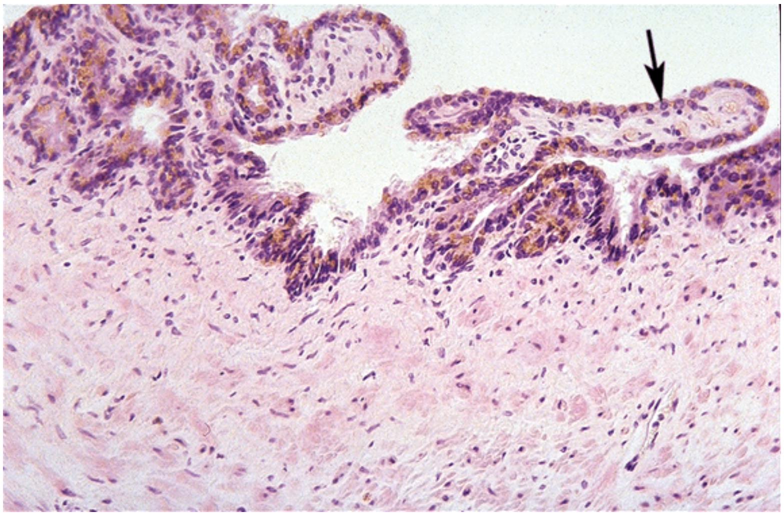

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Figure II-3-62. Seminal vesicle showing mucosal folds lined with pseudostratified columnar epithelium (arrow)

The **prostate** is a collection of 30–50 branched tubuloalveolar glands whose ducts empty into the urethra. The prostate is surrounded by a fibroelastic capsule that is rich in smooth muscle. There are 2 types of glands in the prostate, periurethral submucosal glands and the main prostatic glands in the periphery. Glandular epithelium is pseudostratified columnar with pale, foamy cytoplasm and numerous secretory granules. The products of the secretory granules include acid phosphatase, citric acid, fibrinolysin, and other proteins.

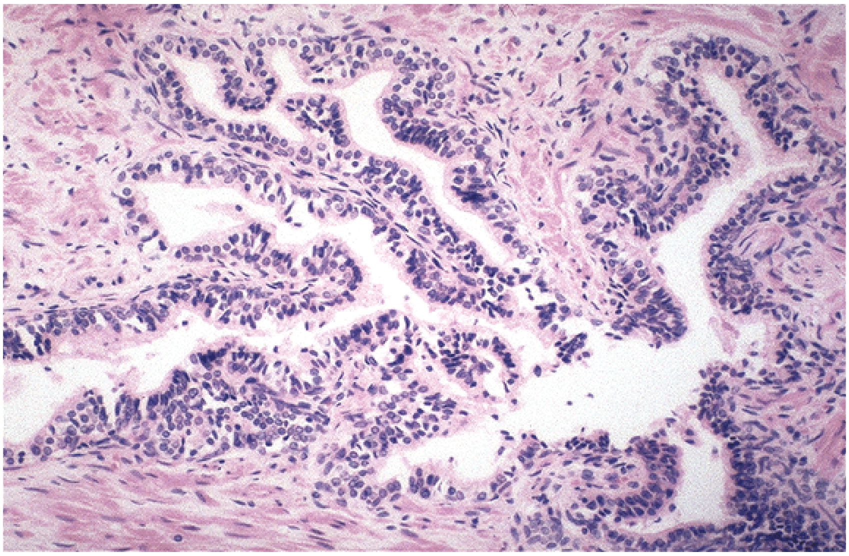

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Figure II-3-63. Prostate with tubuloalveolar glands lined by pseudostratified columnar epithelium

Table II-3-9. Male Reproductive Physiology

Penile Erection
Erection occurs in response to **parasympathetic** stimulation (pelvic splanchnic nerves). **Nitric oxide** is released, causing relaxation of the corpus cavernosum and corpus spongiosum, which allows blood to accumulate in the trabeculae of erectile tissue.
Ejaculation
• **Sympathetic** nervous system stimulation (lumbar splanchnic nerves) mediates movement of mature spermatozoa from the epididymis and vas deferens into the ejaculatory duct. • Accessory glands such as the bulbourethral (Cowper) glands, prostate, and seminal vesicles secrete fluids that aid in sperm survival and fertility. • **Somatic motor efferents** (pudendal nerve) that innervate the bulbospongiosus and ischiocavernous muscles at the base of the penis stimulate the rapid ejection of semen out the urethra during ejaculation. Peristaltic waves in the vas deferens aid in a more complete ejection of semen through the urethra.

Clinical Correlate

- Injury to the bulb of the penis may result in extravasation of urine from the urethra into the superficial perineal space. From this space, urine may pass into the scrotum, into the penis, and onto the anterior abdominal wall.
- Accumulation of fluid in the scrotum, penis, and anterolateral abdominal wall is indicative of a laceration of either the membranous or penile urethra (deep to Scarpa fascia). This can be caused by trauma to the perineal region (saddle injury) or laceration of the urethra during catheterization.

FEMALE REPRODUCTIVE HISTOLOGY

Ovary

The paired **ovaries** have 2 major functions: to produce the female gametes and to produce the steroid hormones which prepare the endometrium for conception and maintain pregnancy should fertilization occur. The ovaries are 3 cm long, 1.5 cm wide, and 1 cm thick. They consist of a **medullary region**, which contains a rich vascular bed with a cellular loose connective tissue, and a **cortical region** where the ovarian follicles reside.

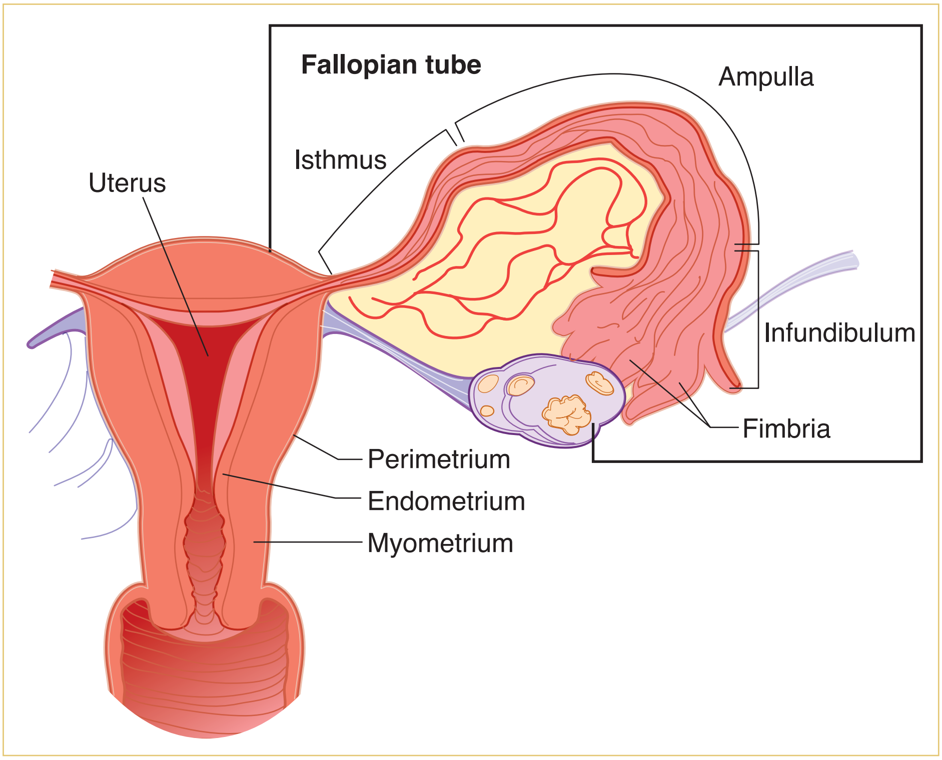

Figure II-3-64. Female Reproductive System

Folliculogenesis and Ovulation

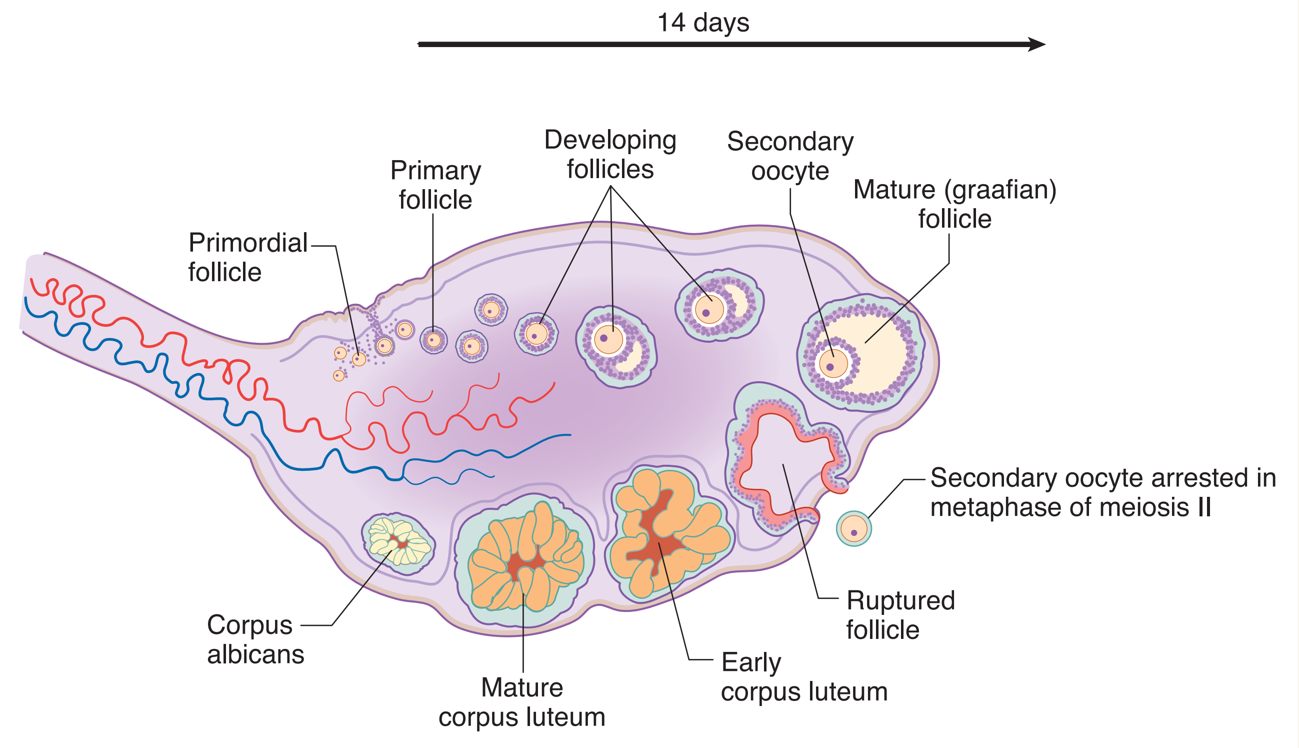

Figure II-3-65. Follicular Development

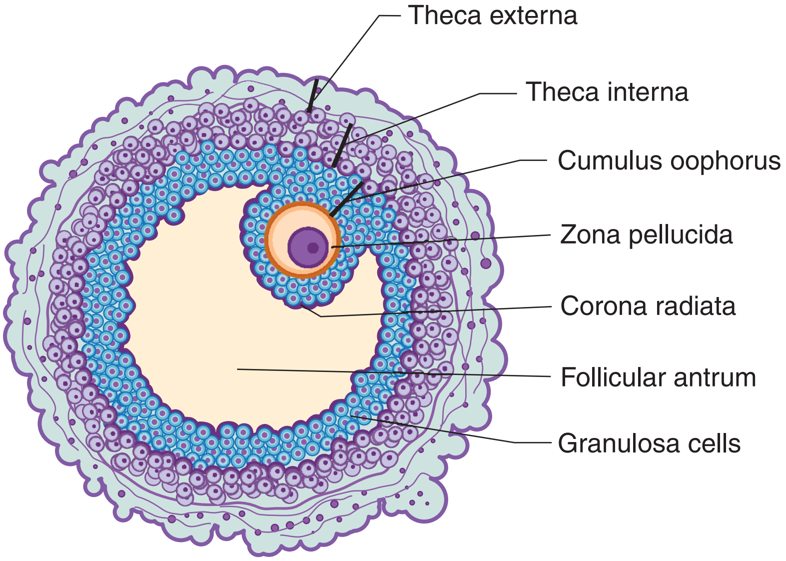

Figure II-3-66. Graafian Follicle

An **ovarian follicle** consists of an oocyte surrounded by one or more layers of follicular cells, the **granulosa cells**. In utero, each ovary initially contains 3 million primordial germ cells. Many undergo atresia as the number of follicles in a normal young adult woman is estimated to be 400,000. A typical woman will ovulate only around 450 ova during her reproductive years. All other follicles (with their oocytes) will fail to mature and will undergo atresia.

Before birth, primordial germ cells differentiate into oogonia that proliferate by mitotic division until they number in the millions. They all enter prophase of the first meiotic division in utero and become arrested (they are now designated as **primordial follicles**). The primordial follicles consist of a primary oocyte surrounded by a single layer of squamous follicular cells, which are joined to one another by desmosomes.

Around the time of sexual maturity, the primordial follicles undergo further growth to become **primary follicles** in which the oocyte is surrounded by 2 or more layers of cuboidal cells. In each menstrual cycle after puberty, several primary follicles enter a phase of rapid growth. The oocyte enlarges and the surrounding follicular cells (now called granulosa cells) proliferate. Gap junctions form between the granulosa cells. A thick layer of glycoprotein called the **zona pellucida** is secreted (probably by both the oocyte and granulosa cells) in the space between the oocyte and granulosa cells. Cellular processes of the granulosa cells and microvilli of the oocyte penetrate the zona pellucida and make contact with one another via gap junctions. Around this time the stroma surrounding the follicle differentiates into a cellular layer called the **theca folliculi**. These cells are separated from the granulosa cells by a thick basement membrane. As development proceeds, 2 zones are apparent in the theca: the **theca interna** (richly vascularized) and the **theca externa** (mostly connective tissue). Cells of the theca interna synthesize androgenic steroids that diffuse into the follicle and are converted to estradiol by the granulosa cells.

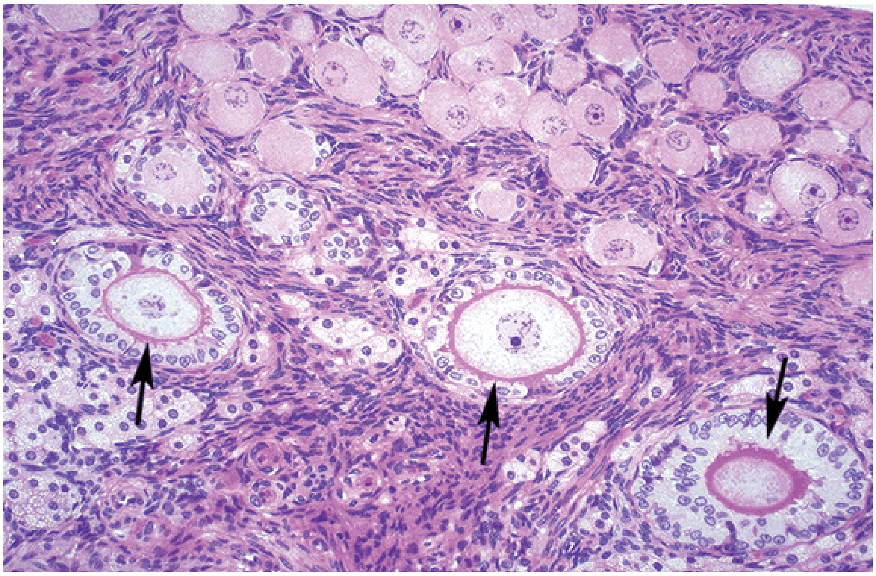

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Figure II-3-67. Ovary

Small primordial follicles are at top and 3 primary follicles (arrows) with cuboidal granulosa cells and a thin zona pellucida are below.

As the follicle grows, follicular **fluid containing** mainly plasma, glycosaminoglycans and steroids accumulates between the cells. The cavities containing the fluid coalesce and form a larger cavity, the **antrum**. At this point, when the antrum is present, the follicles are called **secondary follicles**. The oocyte is at its full size and it is situated in a thickened area of the granulosa called the **cumulus oophorus**.

The mature follicle (**graafian follicle**) completes the first meiotic division (haploid, 2N amount of DNA) just prior to ovulation. The first polar body contains little cytoplasm and remains within the zona pellucida. The Graafian follicle rapidly commences the second meiotic division where it arrests in metaphase awaiting ovulation and fertilization. The second meiotic division is not completed unless fertilization occurs. The fluid filled antrum has greatly enlarged in the Graafian follicle and the cumulus oophorus diminishes leaving the oocyte surrounded by the corona radiata. After ovulation, the corona radiata remains around the ovum where it persists throughout fertilization and for some time during the passage of the ovum through the oviduct.

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Figure II-3-68. Secondary follicle with an antrum

Follicle is surrounded by a corona radiata of granulosa cells (arrow) and by a zona pellucida.

Ovulation occurs approximately mid-cycle and is stimulated by a surge of luteinizing hormone secreted by the anterior pituitary. Ovulation consists of rupture of the mature follicle and liberation of the secondary oocyte (ovum) that will be caught by the infundibulum, the dilated distal end of the oviduct. The ovum remains viable for a maximum of 24 hours. Fertilization most commonly occurs in the ampulla of the oviduct. If not fertilized, the ovum undergoes autolysis in the oviduct.

After ovulation, the wall of the follicle collapses and becomes extensively infolded, forming a temporary endocrine gland called the **corpus luteum**. During this process, the blood vessels and stromal cells invade the previously avascular layer of granulosa cells and the granulosa cells and those of the theca interna

hypertrophy and form lutein cells (granulosa lutein cells and theca lutein cells). The granulosa lutein cells now secrete progesterone and estrogen and the theca lutein cells secrete androstenedione and progesterone. Progesterone prevents the development of new follicles, thereby preventing ovulation.

In the absence of pregnancy the corpus luteum lasts only 10–14 days. The lutein cells undergo apoptosis and are phagocytized by invading macrophages. The site of the corpus luteum is subsequently occupied by a scar of dense connective tissue, the **corpus albicans**.

When pregnancy does occur, human chorionic gonadotropin produced by the placenta will stimulate the corpus luteum for about 6 months and then decline. It continues to secrete progesterone until the end of pregnancy. The corpus luteum of pregnancy is large, sometimes reaching 5 cm in diameter.

Oviducts

The **oviduct** (Fallopian tube) is a muscular tube of ~12 cm in length. One end extends laterally into the wall of the uterus and the other end opens into the peritoneal cavity next to the ovary. The oviduct receives the ovum from the ovary, provides an appropriate environment for its fertilization, and transports it to the uterus. The **infundibulum** opens into the peritoneal cavity to receive the ovum. Finger-like projections (fimbriae) extend from the end of the tube and envelop the ovulation site to direct the ovum to the tube.

Adjacent to the infundibulum is the **ampulla**, where fertilization usually takes place. A slender portion of the oviduct called the **isthmus** is next to the ampulla. The **intramural segment** penetrates the wall of the uterus.

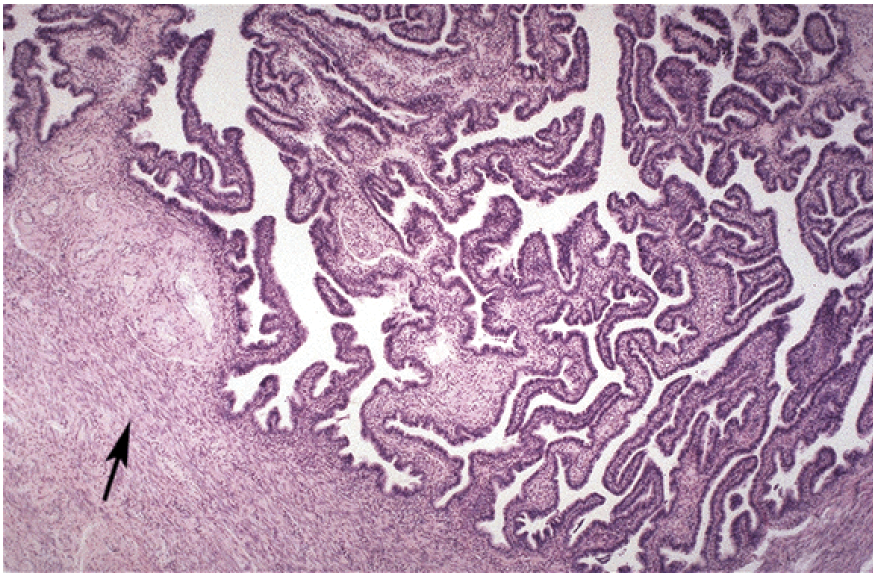

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Figure II-3-69. Oviduct with simple columnar epithelium and underlying layer of smooth muscle (arrow)

The wall of the oviduct has 3 layers: a mucosa, a muscularis, and a serosa composed of visceral peritoneum. The mucosa has longitudinal folds that are most numerous in the ampulla. The epithelium lining the mucosa is simple columnar. Some cells are ciliated and the others are secretory. The cilia beat toward the uterus, causing movement of the viscous liquid film (derived predominantly from the secretory cells) that covers the surface of the cells. The secretion has nutrient and protective functions for the ovum and promotes activation of spermatozoa. Movement of the liquid together with contraction of the muscle layer transports the ovum or fertilized egg (zygote) to the uterus.

Ciliary action is not essential, so women with **immotile cilia syndrome** (**Kartagener's syndrome**) will have a normal tubal transport of the ovum. The muscularis consists of smooth-muscle fibers in an inner circular layer and an outer longitudinal layer.

An **ectopic pregnancy** occurs when the fertilized ovum implants, most commonly in the wall of the ampulla of the oviduct. Partial development proceeds for a time but the tube is too thin and the embryo cannot survive. The vascular placental tissues that have penetrated the thin wall cause brisk bleeding into the lumen of the tube and peritoneal cavity when the tube bursts.

Uterus

The **uterus** is a pear-shaped organ that consists of a fundus which lies above the entrance sites of the oviducts; a **body** (**corpus**) which lies below the entry point of the oviducts and the internal os; a narrowing of the uterine cavity; and a lower cylindrical structure, the **cervix**, which lies below the internal os. The wall of the uterus is relatively thick and has 3 layers. Depending upon the part of the uterus, there is either an outer serosa (connective tissue and mesothelium) or adventitia (connective tissue). The 2 other layers are the **myometrium** (smooth muscle) and the **endometrium** (the mucosa of the uterus).

The myometrium is composed of bundles of smooth-muscle fibers separated by connective tissue. During pregnancy, the myometrium goes through a period of growth as a result of hyperplasia and hypertrophy. The endometrium consists of epithelium and lamina propria containing simple tubular glands that occasionally branch in their deeper portions. The epithelial cells are a mixture of ciliated and secretory simple columnar cells.

The endometrial layer can be divided into 2 zones. The **functionalis** is the part that is sloughed off at menstruation and replaced during each menstrual cycle, and the **basalis** is the portion retained after menstruation that subsequently proliferates and provides a new epithelium and lamina propria. The bases of the uterine glands, which lie deep in the basalis, are the source of the stem cells that divide and migrate to form the new epithelial lining.

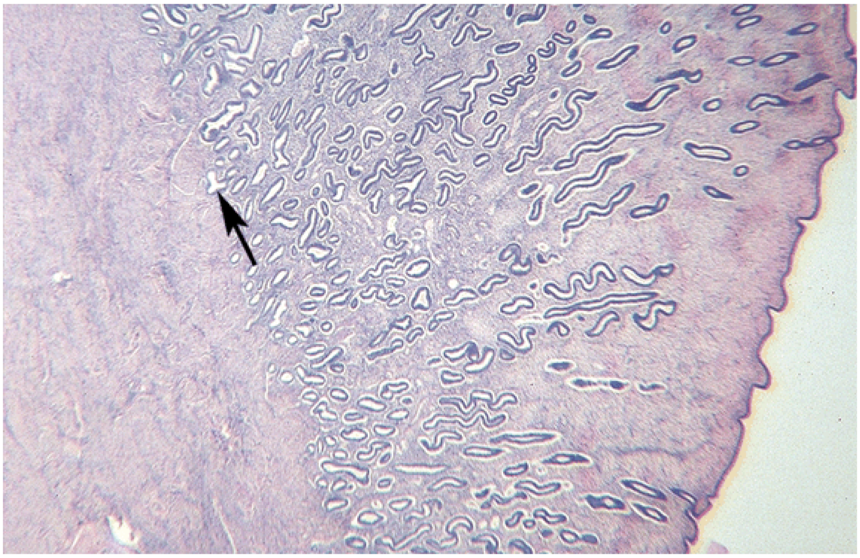

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Figure II-3-70. Uterine wall with endometrium

Simple tubular glands to the right of arrow and myometrium to the left of arrow

Vagina

The wall of the **vagina** has no glands and consists of 3 layers: the mucosa, a muscular layer, and an adventitia. The mucous found in the vagina comes from the glands of the uterine cervix. The epithelium of the mucosa is stratified squamous. This thick layer of cells contains glycogen granules and may contain some keratohyalin. The muscular layer of the vagina is composed of longitudinal bundles of smooth muscle.

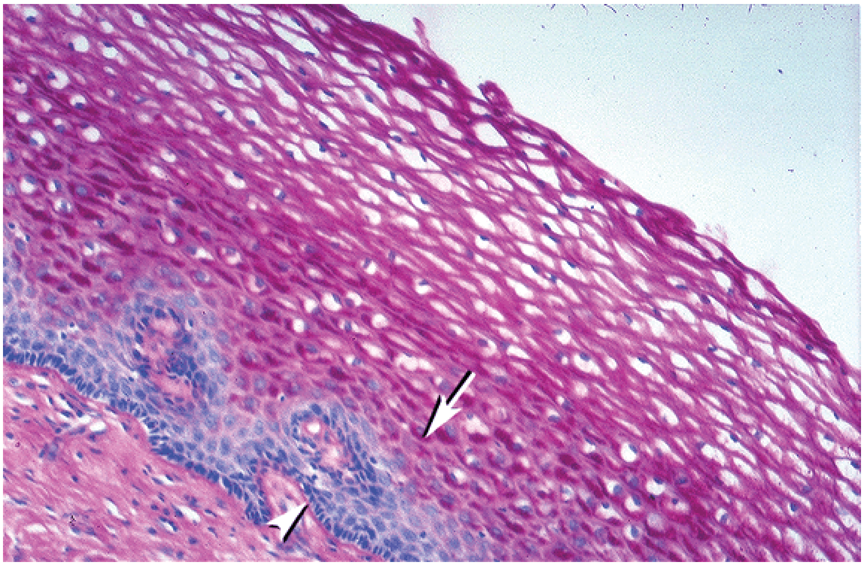

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Figure II-3-71. Vaginal epithelium with vacuolated stratified squamous epithelial cells that contain glycogen, which is removed during histological processing

Clinical Correlate

Breast cancer affects about 9% of women born in the United States. Most of the cancers (carcinomas) arise from epithelial cells of the lactiferous ducts.

Mammary Glands

The mammary glands enlarge significantly during pregnancy as a result of proliferation of alveoli at the ends of the terminal ducts. Alveoli are spherical collections of epithelial cells that become the active milk-secreting structures during lactation. The milk accumulates in the lumen of the alveoli and in the lactiferous ducts. Lymphocytes and plasma cells are located in the connective tissue surrounding the alveoli. The plasma cell population increases significantly at the end of pregnancy and is responsible for the secretion of IgA that confers passive immunity on the newborn.

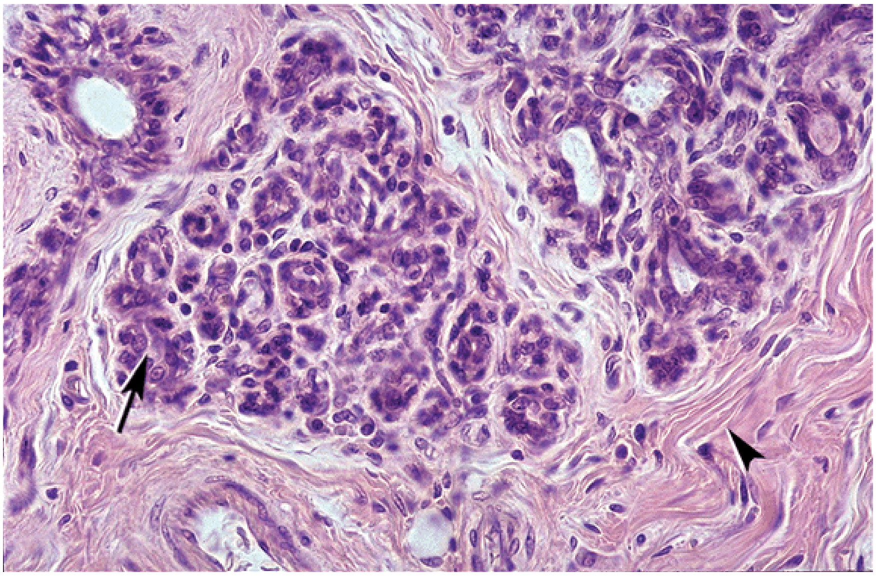

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Figure II-3-72. Breast tissue containing modified mammary gland tissue (arrow) surrounded by dense regular connective tissue (arrowhead)

RADIOLOGY OF THE ABDOMEN AND PELVIS

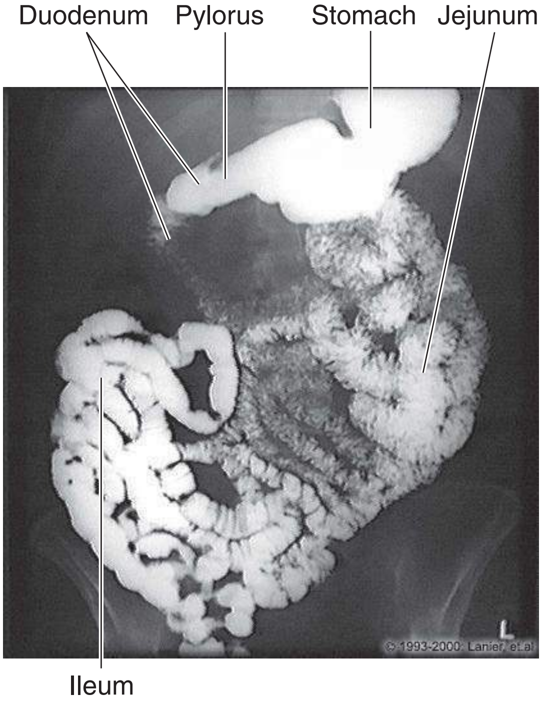

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Figure II-3-73. Abdomen: Upper GI, Small Bowel

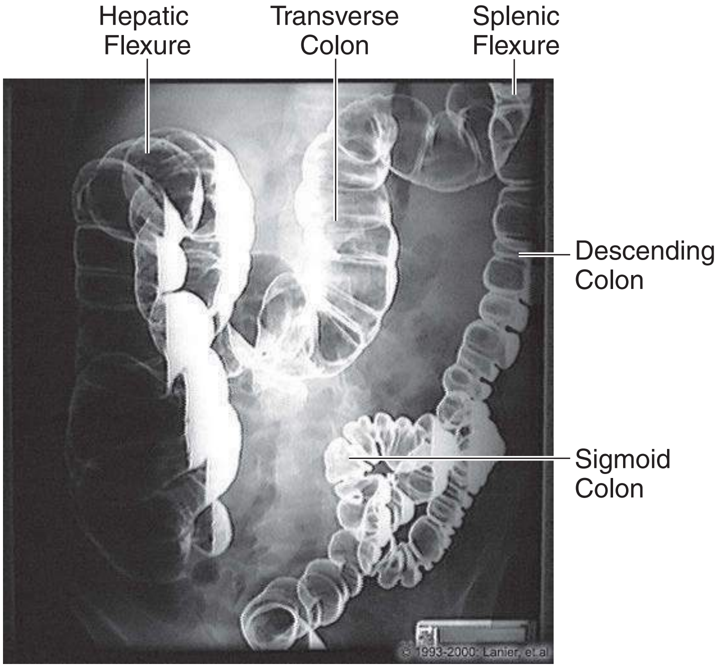

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Figure II-3-74. Abdomen: Barium Enema

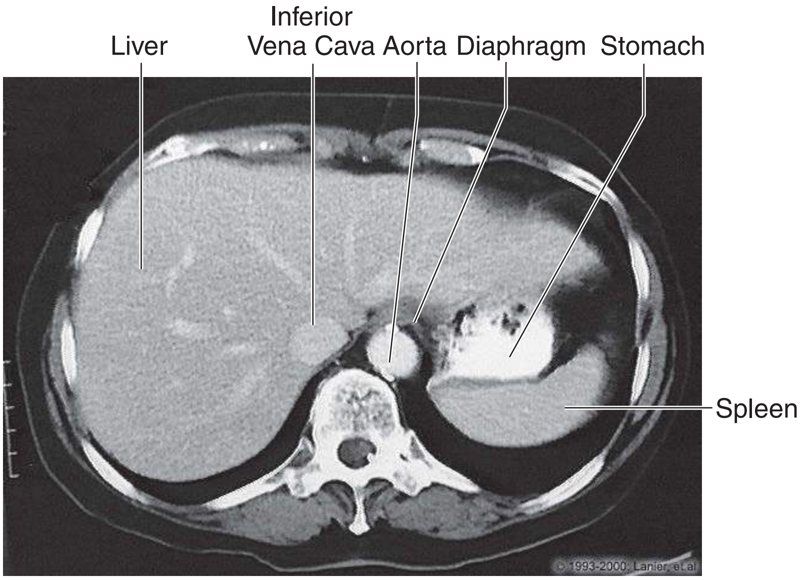

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Figure II-3-75. Abdomen: CT, T11

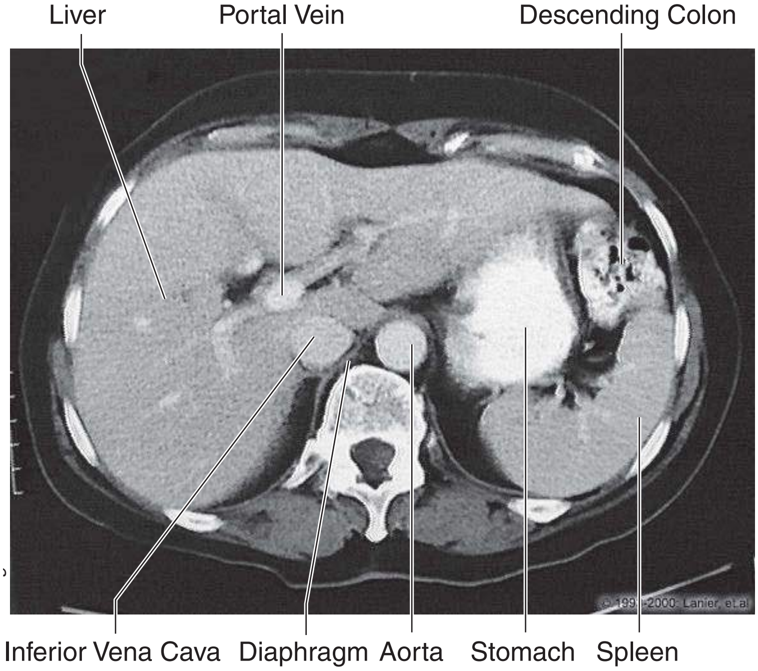

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Figure II-3-76. Abdomen: CT, T12

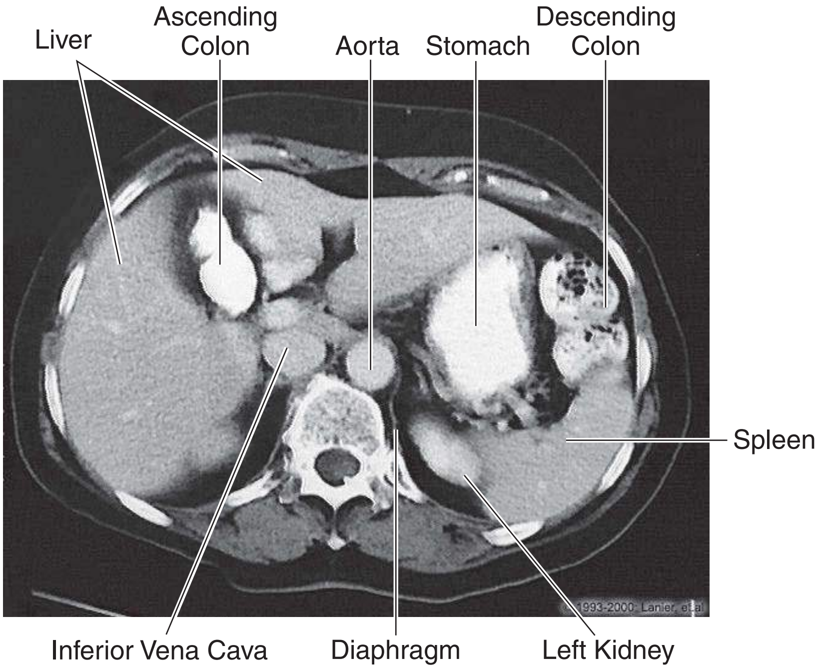

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Figure II-3-77. Abdomen: CT, T12

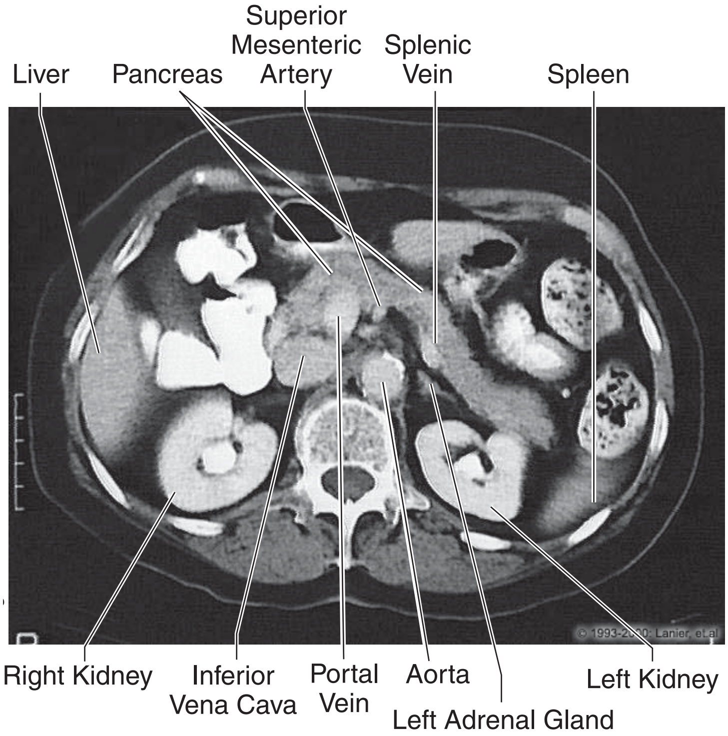

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Figure II-3-78. Abdomen: CT, L1

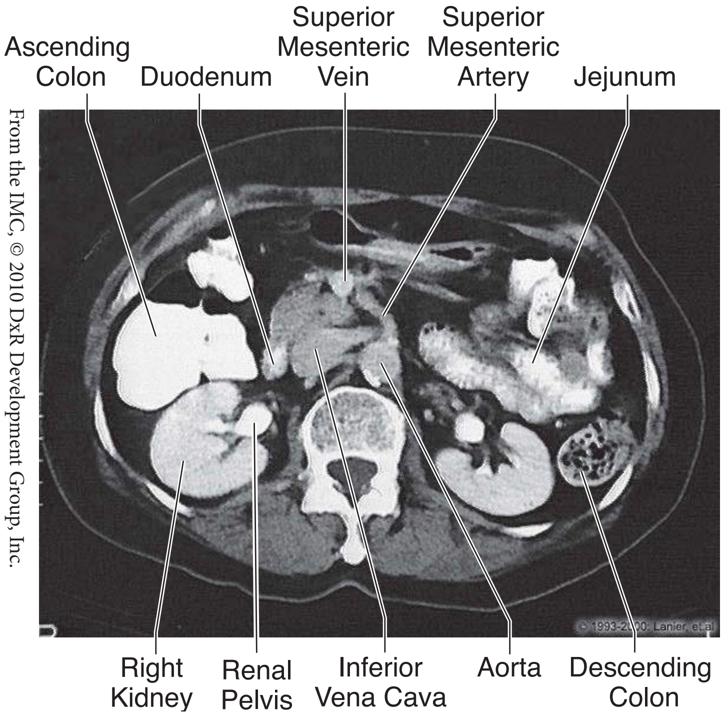

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Figure II-3-79. Abdomen: CT, L2

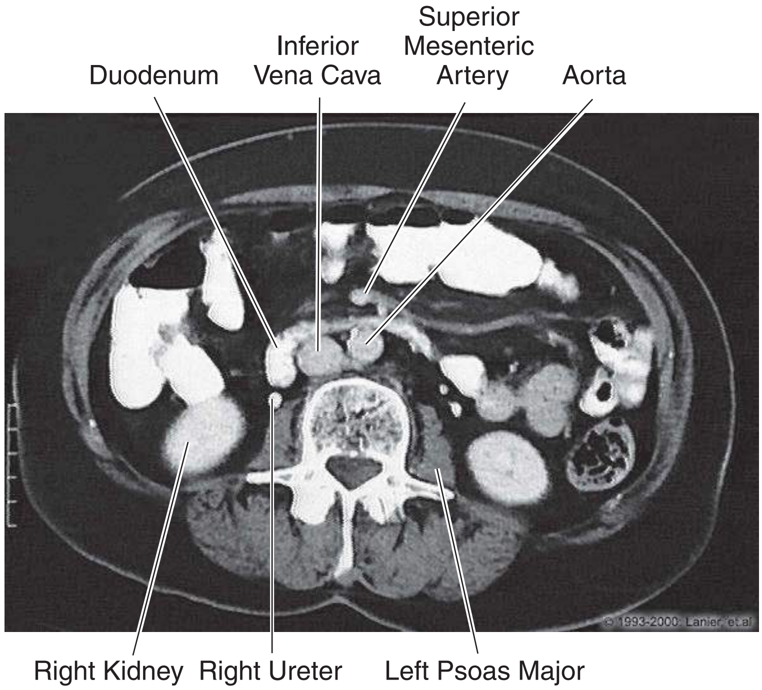

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Figure II-3-80. Abdomen: CT, L3

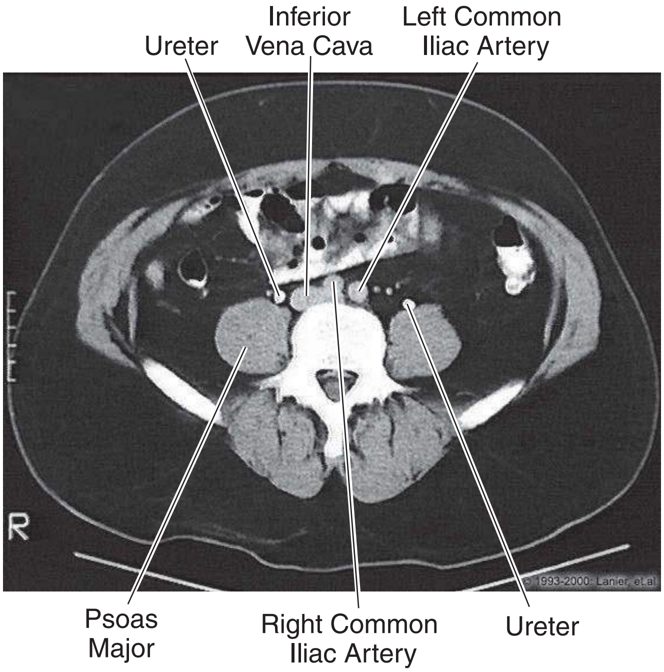

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Figure II-3-81. Abdomen: CT, L4

Upper Limb 4

Learning Objectives

- ❑ Solve problems concerning brachial plexus
- ❑ Answer questions about muscle innervation
- ❑ Solve problems concerning sensory innervation
- ❑ Solve problems concerning nerve injuries
- ❑ Solve problems concerning upper and lower brachial plexus lesions
- ❑ Use knowledge of lesions of branches of the brachial plexus
- ❑ Use knowledge of arterial supply and major anastomoses
- ❑ Solve problems concerning carpal tunnel
- ❑ Interpret scenarios on rotator cuff
- ❑ Use knowledge of radiology

BRACHIAL PLEXUS

The **brachial plexus** provides the motor and sensory innervation to the upper limb and is formed by the ventral rami of C5 through T1 spinal nerves. Five major nerves arise from the brachial plexus:

- The **musculocutaneous, median,** and **ulnar** nerves contain **anterior division fibers** and innervate muscles in the anterior arm, anterior forearm, and palmar compartments that function mainly as flexors.
- The **axillary** and **radial** nerves contain **posterior division fibers** and innervate muscles in the posterior arm and posterior forearm compartments that function mainly as extensors.

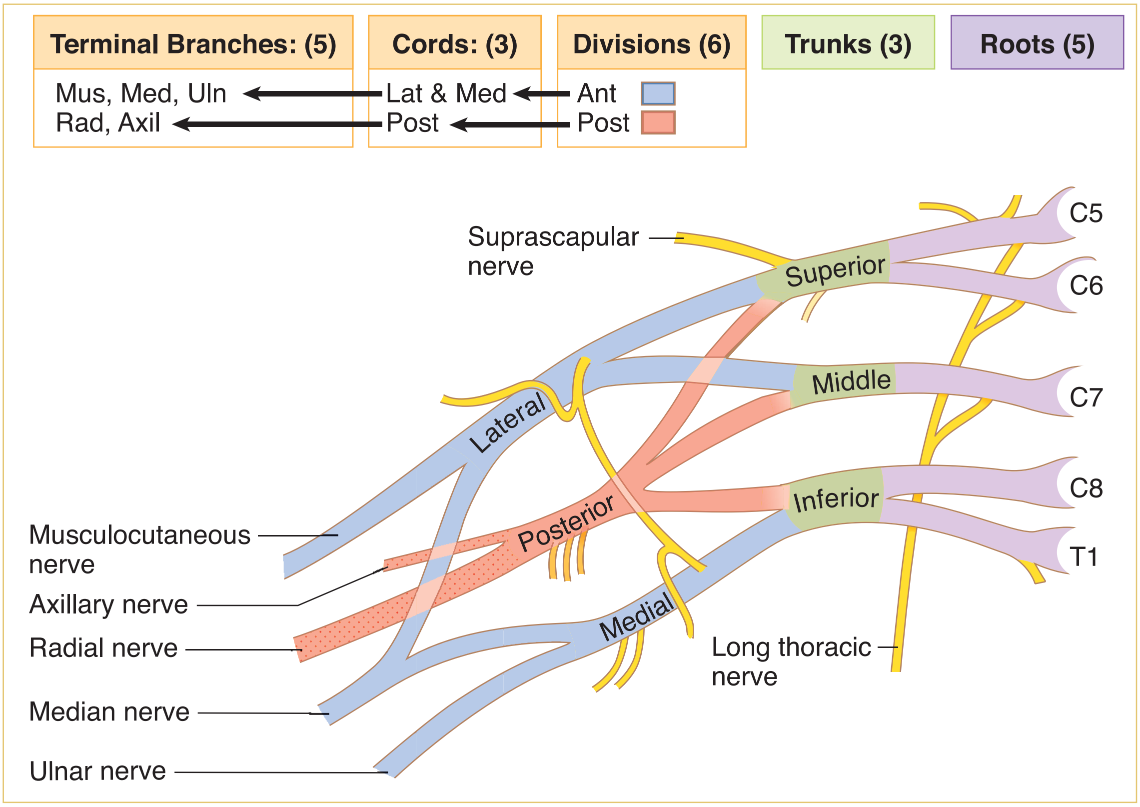

Figure II-4-1. Brachial Plexus

MUSCLE INNERVATION

Terminal Nerves of Upper Limbs

The motor innervation by the 5 terminal nerves of the arm muscles is summarized below.

Table II-4-1. Major Motor Innervations by the 5 Terminal Nerves

Terminal Nerve	Muscles Innervated	Primary Actions
Musculocutaneous nerve C5–6	All the muscles of the **anterior compartment** of the arm	Flex elbow Supination (biceps brachii)
Median nerve C5–T1	**A. Forearm** • **Anterior compartment** except 1.5 muscles by ulnar nerve (flexor carpi ulnaris and the ulnar half of the flexor digitorum profundus)	Flex wrist and all digits Pronation
	B. Hand • **Thenar compartment** • **Central compartment** Lumbricals: Digits 2 and 3	Opposition of thumb Flex metacarpophalangeal (MP) and extend interphalangeal (PIP and DIP) joints of digits 2 and 3
Ulnar nerve C8–T1	**A. Forearm** **Anterior Compartment:** 1 [1/2] muscles not innervated by the median nerve	Flex wrist (weak) and digits 4 and 5
	B. Hand • Hypothenar compartment • **Central compartment** – Interossei muscles: Palmar and Dorsal • Lumbricals: Digits 4 & 5 • Adductor pollicis	{ Dorsal – Abduct digits 2-5 (DAB) Palmar – Adduct digits 2-5 (PAD) Assist Lumbricals in MP flexion and IP extension digits 2–5 } Flex MP and extend PIP & DIP joints of digits 4 and 5 Adduct the thumb
Axillary nerve C5–6	Deltoid Teres minor	Abduct shoulder—15°–110° Lateral rotation of shoulder
Radial nerve C5–T1	**Posterior compartment** muscles of the arm and forearm	Extend MP, wrist, and elbow Supination (supinator muscle)

Collateral Nerves

In addition to the 5 terminal nerves, there are several collateral nerves that arise from the brachial plexus proximal to the terminal nerves (i.e., from the rami, trunks, or cords). These nerves innervate proximal limb muscles (shoulder girdle muscles). Table II-4-2 summarizes the collateral nerves.

Table II-4-2. The Collateral Nerves of the Brachial Plexus

Collateral Nerve	Muscles or Skin Innervated
Dorsal scapular nerve	Rhomboids
Long thoracic nerve	Serratus anterior—protracts and rotates scapula superiorly
Suprascapular nerve C5–6	Supraspinatus—abduct shoulder 0–15° Infraspinatus—laterally rotate shoulder
Lateral pectoral nerve	Pectoralis major
Medial pectoral nerve	Pectoralis major and minor
Upper subscapular nerve	Subscapularis
Middle subscapular (thoracodorsal) nerve	Latissimus dorsi
Lower subscapular nerve	Subscapularis and teres major
Medial brachial cutaneous nerve	Skin of medial arm
Medial antebrachial cutaneous nerve	Skin of medial forearm

Segmental Innervation to Muscles of Upper Limbs

The **segmental innervation** to the muscles of the upper limbs has a **proximal–distal gradient**, i.e., the more proximal muscles are innervated by the higher segments (C5 and C6) and the more distal muscles are innervated by the lower segments (C8 and T1). Therefore, the intrinsic shoulder muscles are innervated by C5 and C6, the intrinsic hand muscles are innervated by C8 and T1, the distal arm and proximal forearm muscles are innervated by C6 and C7, and the more distal forearm muscles are innervated by C7 and C8.

SENSORY INNERVATION

The skin of the palm is supplied by the median and ulnar nerves. The **median** supplies the lateral 3½ digits and the adjacent area of the lateral palm and the thenar eminence. The **ulnar** supplies the medial 1½ digits and skin of the hypothenar eminence. The **radial** nerve supplies skin of the dorsum of the hand in the area of the first dorsal web space, including the skin over the anatomic snuffbox.

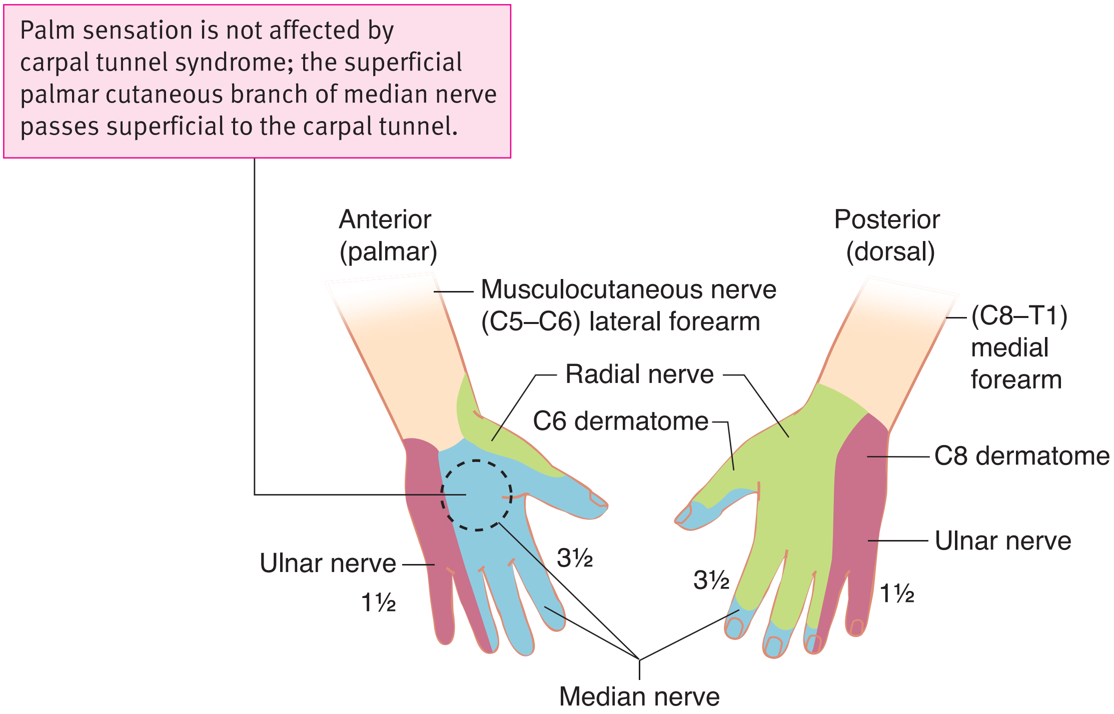

Figure II-4-2. Sensory Innervation of the Hand and Forearm

NERVE INJURIES

Follow clues in the exam questions as to the location of the injury. An injury will manifest in symptoms distal to the site of injury.

Muscle–Nerve Lesions

Without specifically naming all the muscles, assign a function to the various compartments of the limbs.

Example: posterior arm = extension of the forearm and shoulder

List the nerve(s) that innervate those muscles or that area.

Example: posterior arm = radial nerve

You have an area of the limb, a function of the muscles within that area, and a nerve responsible for that function.

Now you can damage a nerve and note what function(s) is lost or weakened.

UPPER AND LOWER BRACHIAL PLEXUS LESIONS

Upper (C5 and C6) Brachial Plexus Lesion: Erb-Duchenne Palsy (Waiter's Tip Syndrome)

- Usually occurs when the head and shoulder are forcibly separated (e.g., accident or birth injury or herniation of disk)

- Trauma will damage **C5** and **C6** spinal nerves (roots) of the upper trunk.
- Primarily affects the **axillary, suprascapular, and musculocutaneous nerves** with the loss of intrinsic muscles of the shoulder and muscles of the anterior arm.
- The arm is medially rotated and adducted at the shoulder: loss of **axillary** and **suprascapular** nerves. The unopposed latissimus dorsi and pectoralis major muscles pull the limb into adduction and medial rotation at the shoulder.
- The forearm is extended and pronated: loss of **musculocutaneous** nerve.
- **Sign is "waiter's tip."**
- Sensory loss on lateral forearm to base of thumb: loss of musculocutaneous nerve

Lower (C8 and T1) Brachial Plexus Lesion: Klumpke's Paralysis

- Usually occurs when the upper limb is forcefully abducted above the head (e.g., grabbing an object when falling, thoracic outlet syndrome or birth injury)
- Trauma will injure the **C8 and T1** spinal nerve roots of inferior trunk.
- Primarily affects the ulnar nerve and the intrinsic muscles of the hand with a weakness of the median innervated muscles of the hand
- Sign is combination of "**claw hand"** and "ape hand" (median nerve).
- May include a Horner syndrome.
- Sensory loss on medial forearm and medial 1½ digits

Table II-4-3. Lesions of Roots of Brachial Plexus

Lesioned Root	C5	C6	C8	T1
Dermatome paresthesia	Lateral border of upper arm	Lateral forearm to thumb	Medial forearm to little finger	Medial arm to elbow
Muscles affected	Deltoid Rotator cuff Serratus anterior Biceps Brachioradialis	Biceps Brachioradialis Brachialis Supinator	Finger flexors Wrist flexors Hand muscles	Hand muscles
Reflex test	—	Biceps tendon	—	—
Causes of lesions	Upper trunk compression	Upper trunk compression	Lower trunk compression	Lower trunk compression

LESIONS OF BRANCHES OF THE BRACHIAL PLEXUS

- Sensory deficits precede motor weakness
- Proximal lesions: more signs

Radial Nerve

Axilla: (Saturday night palsy or using crutches)

- Loss of extension at the elbow, wrist and MP joints
- Weakened supination
- Sensory loss on posterior arm, forearm, and dorsum of thumb
- Distal sign is **"wrist drop."**

Mid-shaft of humerus at radial groove or lateral elbow (lateral epicondyle or radial head dislocation)

- Loss of forearm extensors of the wrist and MP joints
- Weakened supination
- Sensory loss on the posterior forearm and dorsum of thumb
- Distal sign is **"wrist drop."**

Note: Lesions of radial nerve distal to axilla, elbow extension are spared.

Wrist: laceration

- No **motor loss**
- Sensory loss only on dorsal aspect of thumb (first dorsal web space)

Median Nerve

Elbow: (Supracondylar fracture of humerus)

- Weakened wrist flexion (with ulnar deviation)
- Loss of pronation
- Loss of digital flexion of lateral 3 digits resulting in the inability to make a complete fist; sign is "**hand of benediction**"
- Loss of thumb opposition (opponens pollicis muscle); sign is **ape (simian) hand**
- Loss of first 2 lumbricals
- Thenar atrophy (flattening of thenar eminence)
- Sensory loss on palmar surface of the lateral hand and the palmar surfaces of the lateral 3½ digits

Note: A lesion of median nerve at elbow results in the "hand of benediction" and "ape hand."

Wrist: carpal tunnel or laceration

- Loss of thumb opposition (opponens pollicis muscle); sign is **ape or simian hand**
- Loss of first 2 lumbricals
- Thenar atrophy (flattening of thenar eminence)
- Sensory loss on the palmar surfaces of lateral 3½ digits. Note sensory loss on lateral palm may be spared.

Note: Lesions of median nerve at the wrist present without benediction hand and with normal wrist flexion, digital flexion, and pronation.

Ulnar Nerve

Elbow (medial epicondyle), wrist (lacerations), or fracture of hook of hamate

- Loss of hypothenar muscles, third and fourth lumbricals, all interossei and adductor pollicis
- With elbow lesion there is minimal weakening of wrist flexion with radial deviation
- Loss of **abduction** and **adduction of digits 2–5** (interossei muscles)
- Weakened interphalangeal (IP) extension of digits 2–5 (more pronounced in digits 4 and 5)
- Loss of thumb adduction
- Atrophy of the hypothenar eminence
- Sign is **"claw hand."** Note that clawing is greater with a wrist lesion.
- Sensory loss on medial 1½ digits

Axillary Nerve

Fracture of the surgical neck of the humerus or inferior dislocation of the shoulder

- Loss of abduction of the arm to the horizon
- Sensory lost over the deltoid muscle

Musculocutaneous Nerve

- Loss of elbow flexion and weakness in supination
- Loss of sensation on lateral aspect of the forearm

Long Thoracic Nerve

- Often damaged during a radical mastectomy or a stab wound to the lateral chest (nerve lies on superficial surface of serratus anterior muscle).

- Loss of abduction of the arm above the horizon to above the head
- Sign of **"winged scapula"**; patient unable to hold the scapula against the posterior thoracic wall

Suprascapular Nerve

- Loss of shoulder abduction between 0 and 15 degrees (supraspinatus muscle)
- Weakness of lateral rotation of shoulder (infraspinatus muscle)

Table II-4-4. Effects of Lesions to Branches of the Brachial Plexus

Lesioned Nerve	Axillary (C5, C6)	Musculo-cutaneous (C5, C6, C7)	Radial (C5, C6, C7, C8)	Median (C6, C7, C8, T1)	Ulnar (C8, T1)
Altered sensation	Lateral arm	Lateral forearm	Dorsum of hand over first dorsal interosseous and anatomic snuffbox	Lateral 3½ digits; lateral palm	Medial 1½ digits; medial palm
Motor weakness	Abduction at shoulder	Flexion of forearm Supination	Wrist extension Metacarpo-phalangeal extension Supination	Wrist flexion Finger flexion Pronation Thumb opposition	Wrist flexion Finger spreading Thumb adduction Finger extension
Common sign of lesion	—	—	Wrist drop	Ape hand Hand of benediction Ulnar deviation at wrist	Claw hand Radial deviation at wrist
Causes of lesions	Surgical neck fracture of humerus Dislocated humerus	Rarely lesioned	Saturday night palsy Midshaft fracture of humerus Subluxation of radius Dislocated humerus	Carpal tunnel compression Supracondylar fracture of humerus Pronator teres syndrome	Fracture of medial epicondyle of humerus Fracture of hook of hamate Fracture of clavicle

ARTERIAL SUPPLY AND MAJOR ANASTOMOSES

Arterial Supply to the Upper Limb

Subclavian artery

Branch of brachiocephalic trunk on the right and aortic arch on the left.

Axillary artery

- From the first rib to the posterior edge of the teres major muscle
- Three major branches:
  - Lateral thoracic artery—supplies mammary gland; runs with long thoracic nerve
  - Subscapular artery—collateral to shoulder with suprascapular branch of subclavian artery
  - Posterior humeral circumflex artery—at surgical neck with axillary nerve

Brachial artery

Profunda brachii artery with radial nerve in radial groove—at midshaft of humerus

Radial artery

Deep palmar arch

Ulnar artery

- Common interosseus artery
- Superficial palmar arch

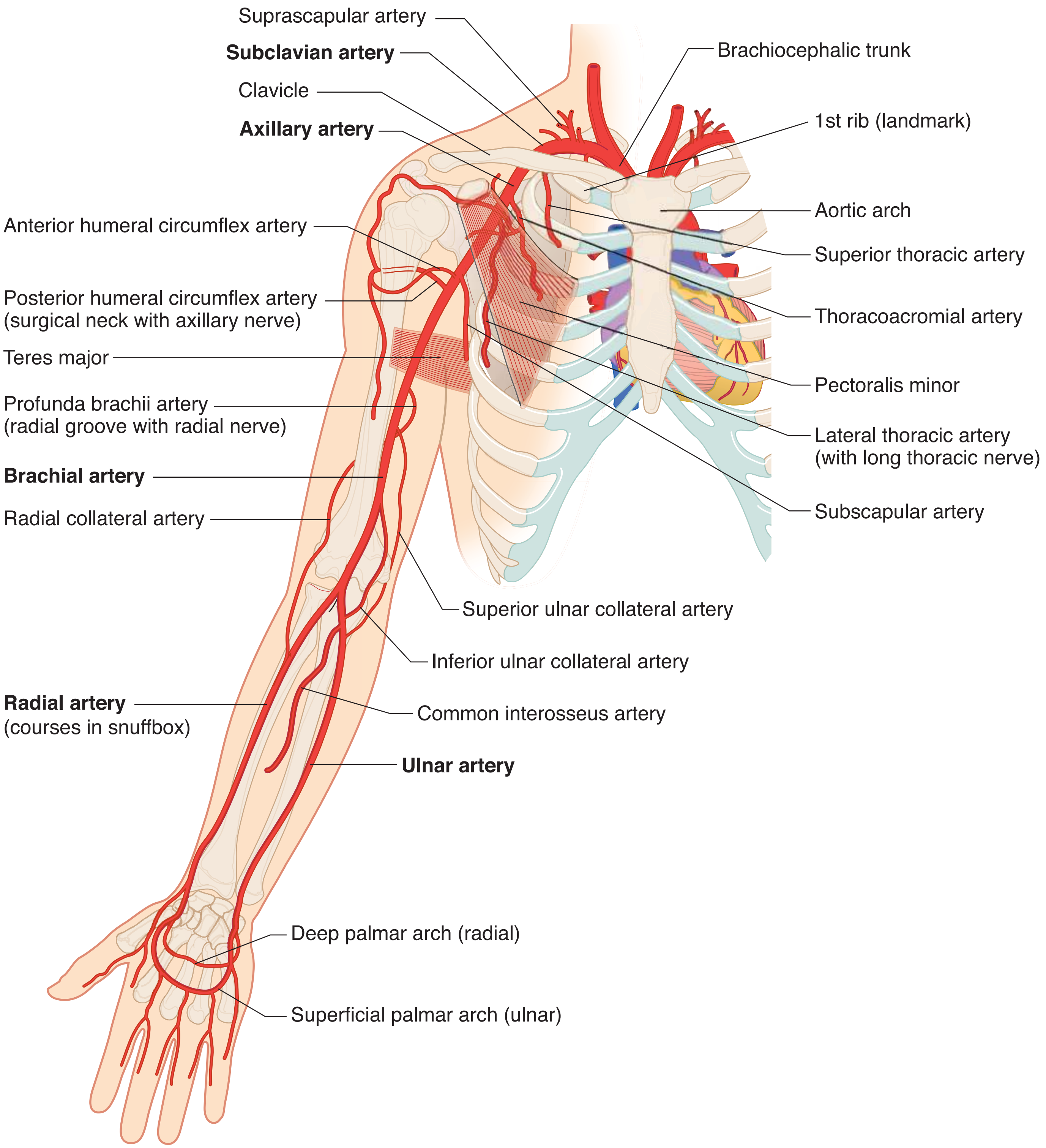

Figure II-4-3. Arterial Supply to the Upper Limb

Collateral Circulation

Shoulder

Subscapular branch of axillary and suprascapular branch of subclavian arteries

Hand

Superficial and deep palmar arches

CARPAL TUNNEL

The carpal tunnel is the fibro-osseous tunnel located on the ventral aspect of the wrist. The tunnel is bounded **anteriorly** by the **flexor retinaculum** and **posteriorly** by the proximal row of **carpal bones (lunate)**.

- The carpal tunnel transmits 9 tendons and the radial and ulnar bursae (4 tendons of the **flexor digitorum superficialis**, 4 tendons of the **flexor digitorum profundus**, and the tendon of the **flexor pollicis longus**) and the **median nerve**.
- There are **no** blood vessels or any branches of the radial or ulnar nerves in the carpal tunnel.

Carpal Tunnel Syndrome

Entrapment of the median nerve and other structures in the carpal tunnel due to any condition that reduces the space results in **carpal tunnel syndrome**. The median nerve is the only nerve affected and the patient will present with atrophy of the thenar compartment muscles and weakness of the thenar muscles (opposition of the thumb—**ape hand**).

There is also **sensory loss** and numbness on the palmar surfaces of the **lateral 3½ digits**. The skin on the lateral side of the palm (thenar eminence) is spared because the **palmar cutaneous branch of the median nerve** which supplies the lateral palm, enters the hand **superficial** to the flexor retinaculum and does not course through the carpal tunnel.

Clinical Correlate

Carpal tunnel syndrome compresses the median nerve.

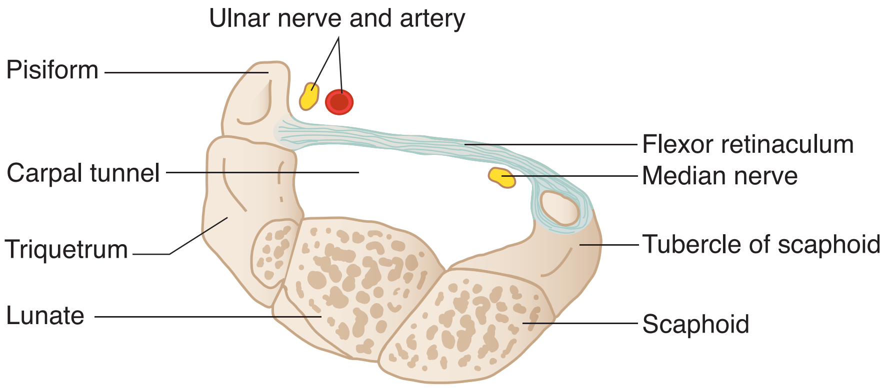

Figure II-4-4. Carpal Tunnel at Proximal Row of Carpal Bones

ROTATOR CUFF

The tendons of rotator cuff muscles strengthen the glenohumeral joint and include the **supraspinatus, infraspinatus, teres minor,** and **subscapularis** (**SITS**) muscles. The tendons of the muscles of the rotator cuff may become torn or inflamed.

The tendon of the **supraspinatus** is most commonly affected. Patients with rotator cuff tears experience pain anteriorly and superiorly to the glenohumeral joint during abduction

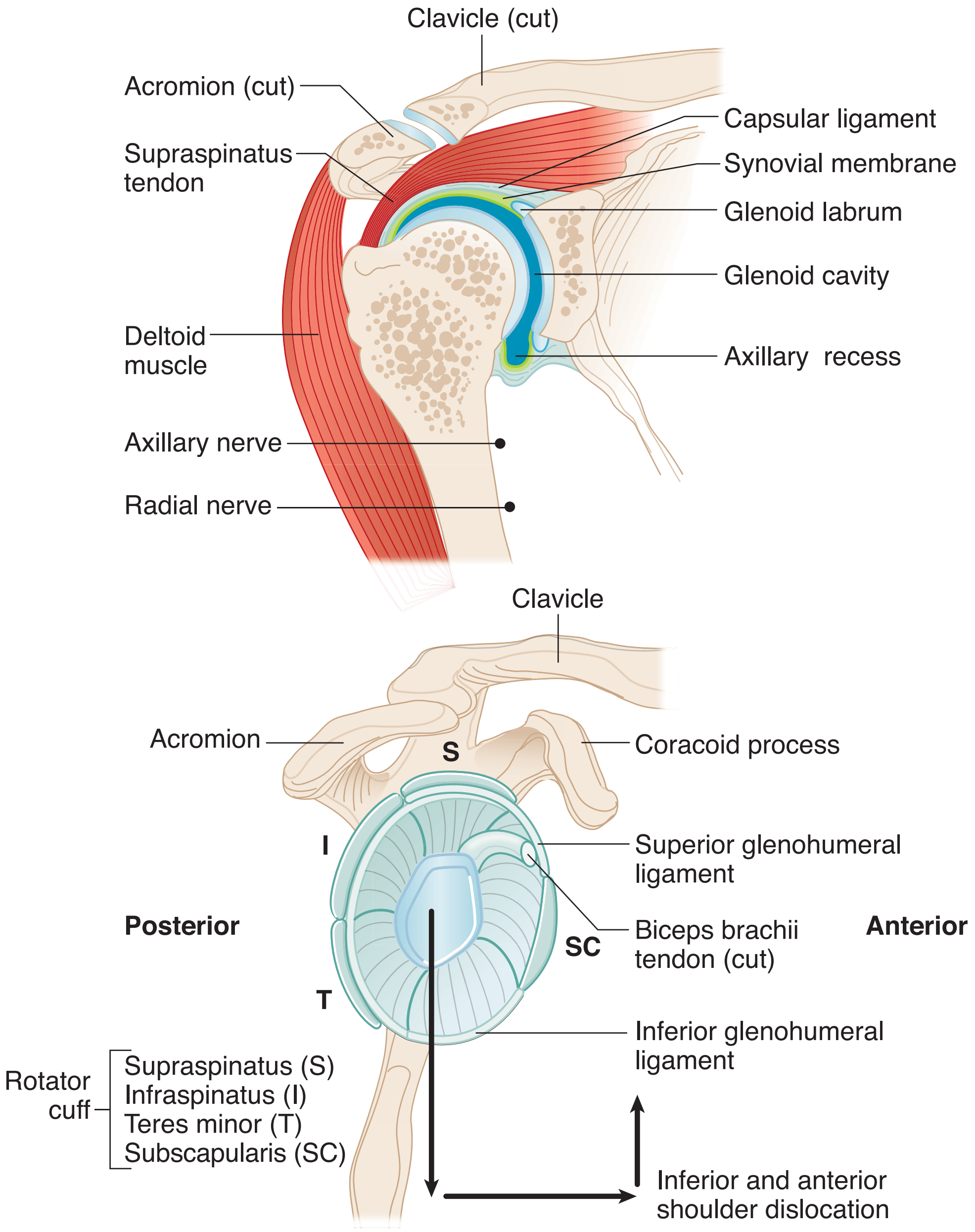

Figure II-4-5. Rotator Cuff

Clinical Correlate

Humeral Head Dislocation

Dislocation of the humeral head from the glenohumeral joint typically occurs through the inferior portion of the joint capsule where the capsule is the slackest and is not reinforced by a rotator cuff tendon (Figure II-4-5). After inferior dislocation, the humeral head is pulled superiorly and comes to lie anterior to the glenohumeral joint.

Dislocation may injure the **axillary** or **radial** nerve.

Clinical Correlate

A rupture or tear of the rotator cuff follows chronic use of the shoulder or a fall with an abducted upper limb. The **supraspinatus muscle** is the most frequently damaged muscle of the rotator cuff.

Clinical Correlate

Humeral Surgical Neck Fracture

The axillary nerve accompanies the posterior humeral circumflex artery as it passes around the surgical neck of the humerus. A fracture in this area could lacerate both the artery and nerve.

Mid-Shaft (Radial Groove) Humeral Fracture

The radial nerve accompanies the profunda brachii artery. Both could be damaged as a result of a mid-shaft humeral fracture.

RADIOLOGY

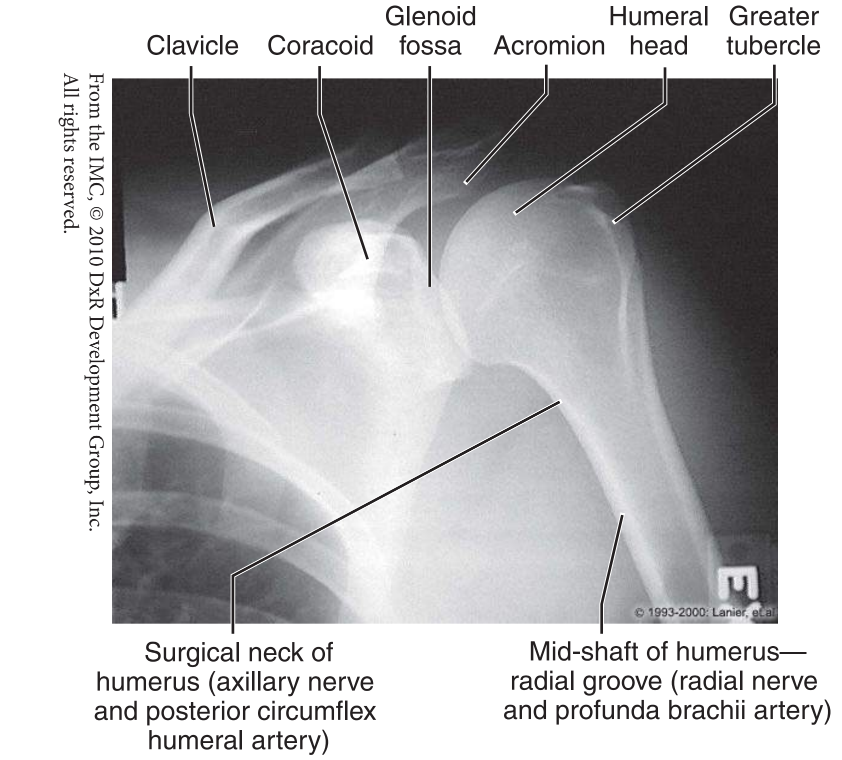

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Figure II-4-6. Upper Extremities: Anteroposterior View of Shoulder (External Rotation)

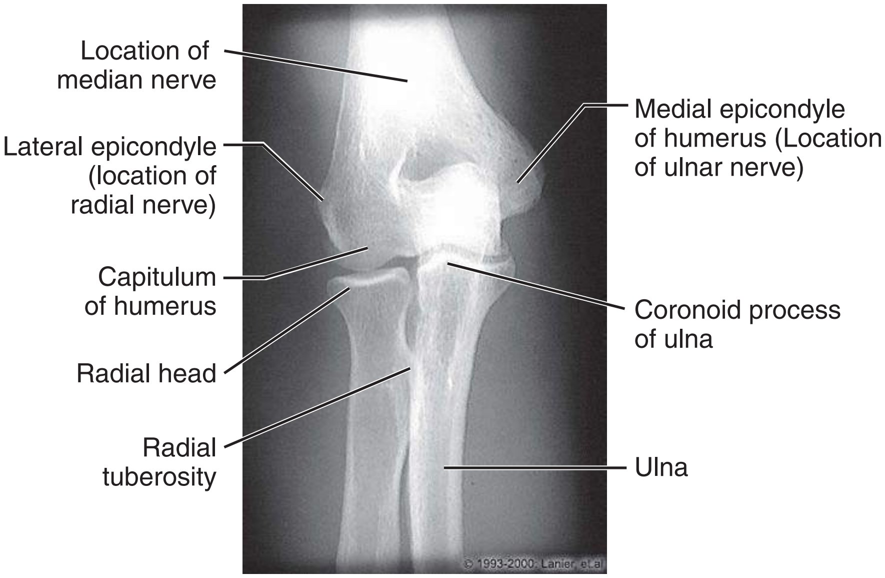

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Figure II-4-7. Upper Extremities: Anteroposterior View of Elbow

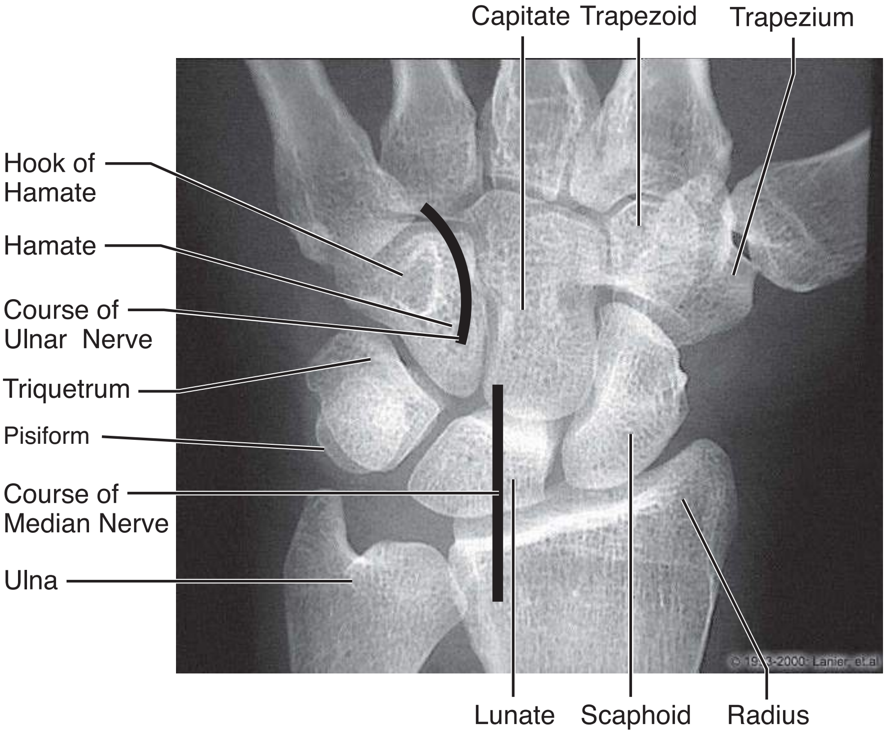

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Figure II-4-8. Upper Extremities: Posteroanterior View of Wrist

Clinical Correlate

The **scaphoid** is the most frequently fractured of the carpal bones. This fracture may separate the proximal head of the scaphoid from its blood supply (which enters the bone at the distal head) and may result in **avascular necrosis** of the proximal head.

The **lunate** is the most commonly dislocated carpal bone (it dislocates anteriorly into the carpal tunnel and may compress the median nerve).

Clinical Correlate

- **Carpal tunnel syndrome** results from compression of the median nerve within the tunnel.
- A fall on the outstretched hand may **fracture the hook of the hamate**, which may damage the ulnar nerve as it passes into the hand.

Lower Limb 5

Learning Objectives

- ❑ Explain information related to lumbosacral plexus
- ❑ Solve problems concerning nerve injuries and abnormalities of gait
- ❑ Demonstrate understanding of arterial supply and major anastomoses
- ❑ Use knowledge of femoral triangle
- ❑ Demonstrate understanding of hip
- ❑ Explain information related to knee joint
- ❑ Use knowledge of ankle joint
- ❑ Solve problems concerning radiology

LUMBOSACRAL PLEXUS

The **lumbosacral plexus** provides the motor and sensory innervation to the lower limb and is formed by ventral rami of the L2 through S3 spinal nerves.

The major nerves of the plexus are:

- **Femoral nerve**: posterior divisions of L2 through L4
- **Obturator nerve**: anterior divisions of L2 through L4
- **Tibial nerve**: anterior divisions of L4 through S3
- **Common fibular nerve**: posterior divisions of L4 through S2
- **Superior gluteal nerve**: posterior divisions of L4 through S1
- **Inferior gluteal nerve**: posterior divisions of L5 through S2

The tibial nerve and common fibular nerve travel together through the gluteal region and thigh in a common connective tissue sheath; together, they are called the **sciatic nerve**.

The common fibular nerve divides in the proximal leg into the **superficial and deep fibular nerve**.

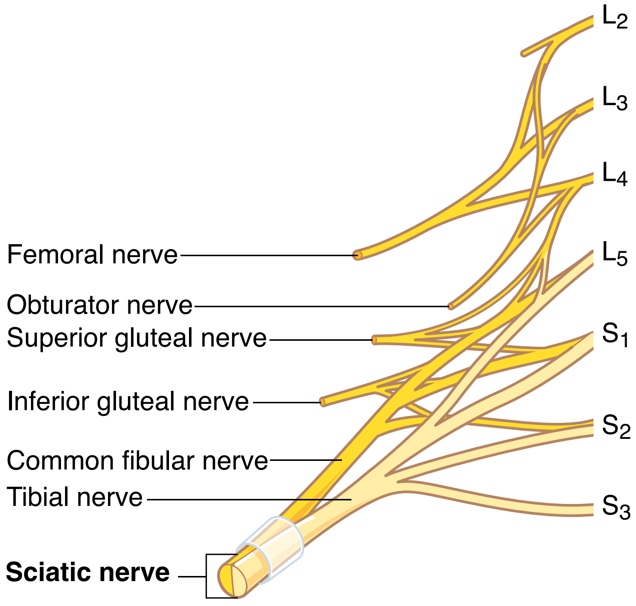

Figure II-5-1. Lumbosacral Plexus

Terminal Nerves of Lumbosacral Plexus

The terminal nerves of the lumbosacral plexus are described below.

Table II-5-1. Terminal Nerves of Lumbosacral Plexus

Terminal Nerve	Origin	Muscles Innervated	Primary Actions
Femoral nerve	L2–L4 posterior divisions	Anterior compartment of thigh (quadriceps femoris, sartorius, pectineus)	Extend knee Flex hip
Obturator nerve	L2–L4 anterior divisions	Medial compartment of thigh (gracilis, adductor longus, adductor brevis, anterior portion of adductor magnus)	Adduct thigh Medially rotate thigh
Tibial nerve	L4–S3 anterior divisions	Posterior compartment of thigh (semimembranosus, semitendinosus, long head of biceps femoris, posterior portion of adductor magnus) Posterior compartment of leg (gastrocnemius, soleus, flexor digitorum longus, flexor hallucis longus, tibialis posterior) Plantar muscles of foot	Flex knee Extend thigh Plantar flex foot (S1–2) Flex digits Inversion
Common fibular nerve	L4–S2 posterior divisions	Short head of biceps femoris	Flex knee
Superficial fibular nerve		Lateral compartment of leg (fibularis longus, fibularis brevis)	Eversion
Deep fibular nerve		Anterior compartment of leg (tibialis anterior, extensor hallucis, extensor digitorum, fibularis tertius)	Dorsiflex foot (L4–5) Extend digits Inversion

Collateral Nerves of Lumbosacral Plexus

The collateral nerves of the lumbosacral plexus (to the lower limb) are summarized below.

Table II-5-2. Collateral Nerves of Lumbosacral Plexus

Collateral Nerve	Origin	Muscles or Skin Innervated	Primary Actions
Superior gluteal nerve	L4–S1 posterior divisions	Gluteus medius, gluteus minimus, tensor fasciae latae	Stabilize pelvis Abduct hip
Inferior gluteal nerve	L5–S2 posterior divisions	Gluteus maximus	Extension of hip Lateral rotation of thigh
Nerve to superior gemellus and obturator internus	L5–S2 posterior divisions	Superior gemellus, obturator internus	Lateral rotation of thigh
Nerve to inferior gemellus and quadratus femoris	L4–S1 posterior divisions	Inferior gemellus, quadratus femoris	Lateral rotation of thigh
Lateral femoral cutaneous nerve	L2–L3 posterior divisions	Skin of anterolateral thigh	—
Posterior femoral cutaneous nerve	S1–S2 posterior divisions and S2–S3 anterior divisions	Skin of posterior thigh	—

Segmental Innervation to Muscles of Lower Limb

The **segmental innervation** to the muscles of the lower limb has a **proximal–distal gradient,** i.e., the more proximal muscles are innervated by the higher segments and the more distal muscles are innervated by the lower segments.

- Muscles that cross the **anterior side of the hip** are innervated by **L2 and L3**
- Muscles that cross the **anterior side of the knee** are innervated by **L3 and L4**
- Muscles that cross the **anterior side of the ankle** are innervated by **L4 and L5 (dorsiflexion)**
- Muscles that cross the **posterior side of the hip** are innervated by **L4 and L5**
- Muscles that cross the **posterior side of the knee** are innervated by **L5 and S1**
- Muscles that cross the **posterior side of the ankle** are innervated by **S1 and S2 (plantar flexion)**

NERVE INJURIES AND ABNORMALITIES OF GAIT

Superior Gluteal Nerve

- Weakness in abduction of the hip
- Impairment of gait; patient cannot keep pelvis level when standing on one leg.
- Sign is "Trendelenburg gait."

Inferior Gluteal Nerve

- Weakened hip extension
- Difficulty rising from a sitting position or climbing stairs

Femoral Nerve

- Weakened hip flexion
- Weakened extension of the knee
- Sensory loss on the anterior thigh, medial leg, and foot

Obturator Nerve

- Loss of adduction of the thigh as well as sensory loss on medial thigh

Sciatic Nerve

- Weakened extension of the thigh
- Loss of flexion of the knee
- Loss of all functions below the knee
- Sensory loss on the posterior thigh, leg (except medial side), and foot

Tibial nerve only

- Weakness in flexion of the knee
- Weakness in plantar flexion
- Weakened inversion
- Sensory loss on the leg (except medial) and plantar foot

Common fibular nerve (neck of fibula)

Produces a combination of deficits of lesions of the deep and superficial fibular nerves

Deep fibular nerve

- Weakened inversion
- Loss of extension of the digits

Clinical Correlate

The **common fibular nerve** crosses the lateral aspect of the knee at the neck of the fibula, where it is the most frequently damaged nerve of the lower limb. Patients will present with loss of dorsiflexion at the ankle (**foot drop**), loss of **eversion**, and sensory loss on the lateral surface of the leg and the dorsum of the foot.

Clinical Correlate

The **common fibular nerve** may be compressed by the **piriformis muscle** when the nerve passes through the piriformis instead of inferior to the muscle with the tibial nerve. **Piriformis syndrome** results in motor and sensory loss to the lateral and anterior compartments of the leg.

- Loss of dorsiflexion ("foot drop")
- Sensory loss limited to skin of the first web space between the great and second toes

Superficial fibular nerve

- Loss of eversion of the foot
- Sensory loss on anterolateral leg and dorsum of the foot, except for the first web space

Sensory Innervation of the Lower Leg and Foot

- The lateral leg and the dorsum of the foot are supplied mainly by the **superficial fibular nerve**, with the exception of the first dorsal web space, which is supplied by the **deep fibular nerve**.
- The sole of the foot is supplied by the **lateral** and **medial plantar branches** of the **tibial nerve**.
- The sural nerve (a combination of both peroneal and tibial branches) supplies the posterior leg and lateral side of the foot.
- The **saphenous nerve** (a branch of the femoral nerve) supplies the medial leg and medial foot.

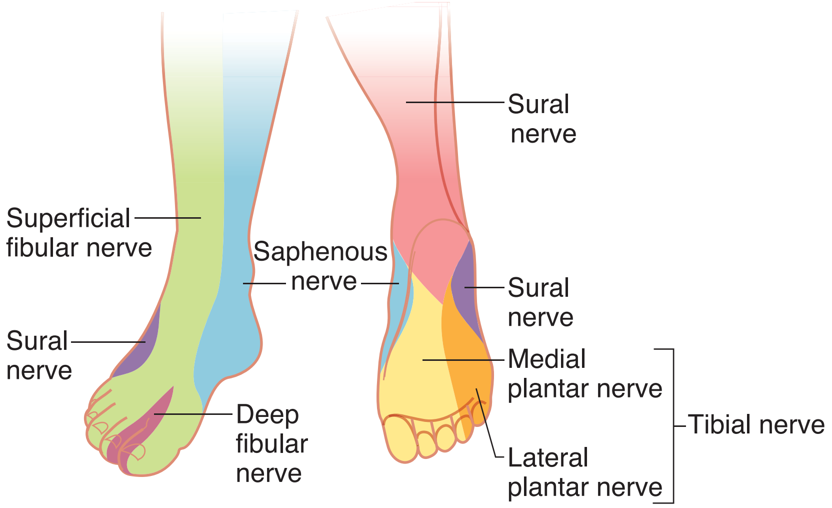

Figure II-5-2. Sensory Innervation of the Lower Leg and Foot

Clinical Correlate

The **sciatic nerve** is often damaged following posterior hip dislocation. A complete sciatic nerve lesion results in sensory and motor deficits in the posterior compartment of the thigh and all functions below the knee.

ARTERIAL SUPPLY AND MAJOR ANASTOMOSES

The obturator artery supplies the medial compartment of the thigh.

- **External iliac artery**
- **Femoral artery**
  - Profunda femoris artery
    - Medial circumflex femoral artery—supplies head of femur (avascular necrosis)
    - Lateral circumflex femoral artery
    - Perforating arteries—supplies posterior compartment of thigh
- **Popliteal artery:** supplies knee joint
  - Anterior tibial artery: courses with deep fibular nerve in anterior compartment of leg
    - Dorsalis pedis artery: pulse on dorsum of foot lateral to extensor hallucis longus tendon; used to note quality of blood supply to foot
  - Posterior tibial artery: courses with tibial nerve in posterior compartment of leg and passes posterior to the medial malleolus
    - Fibular artery: supplies lateral compartment of leg
    - Plantar arterial arch
    - Lateral plantar artery
    - Medial plantar artery

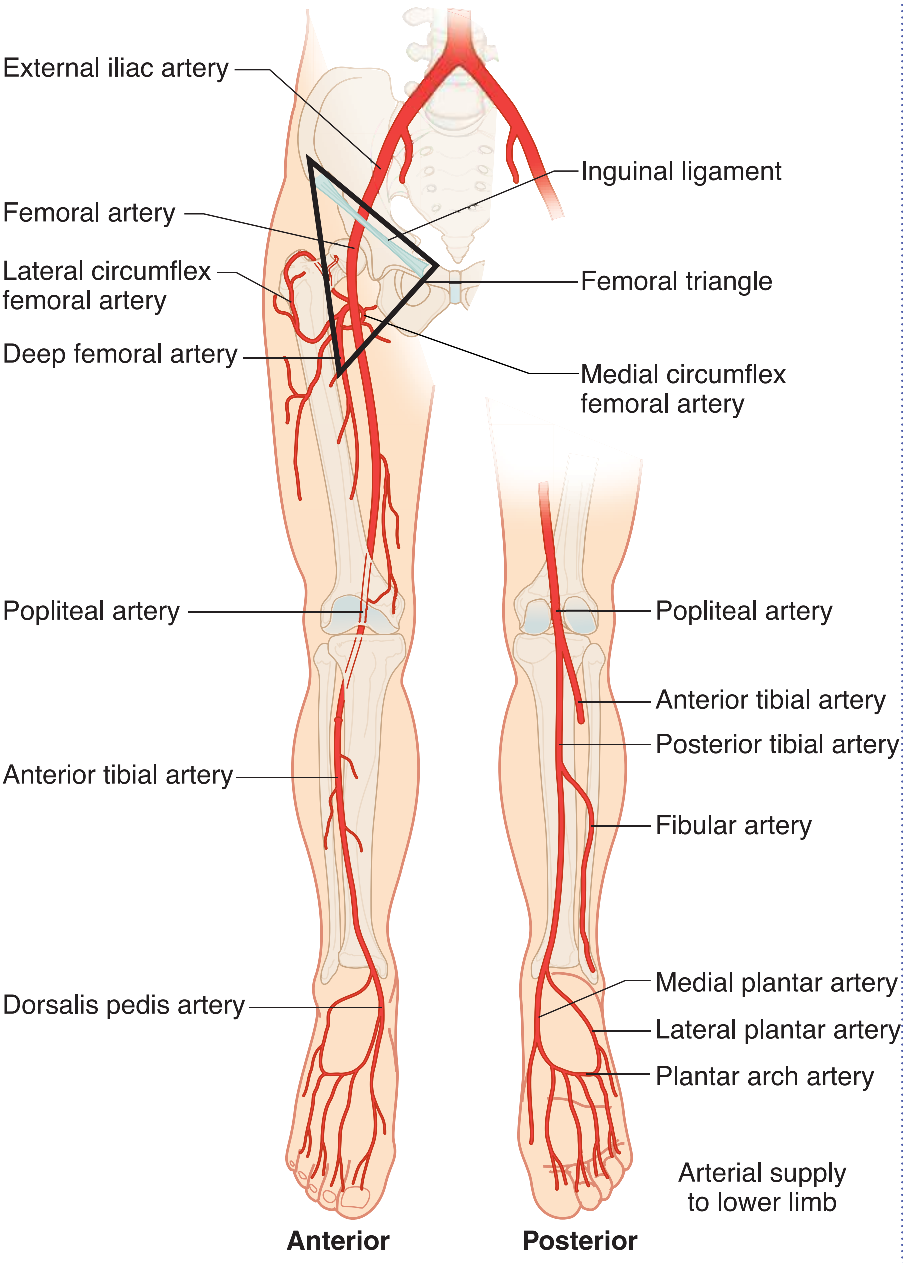

Figure II-5-3. Arterial Supply to Lower Limb

FEMORAL TRIANGLE

The femoral triangle is bounded by the inguinal ligament, and the sartorius and adductor longus muscles. Within the triangle are the femoral sheath (containing the femoral artery and vein and canal) and the femoral nerve (which is outside of the femoral sheath).

Passing under the inguinal ligament (from lateral to medial) are the femoral nerve, femoral artery, femoral vein, an empty space within the femoral sheath called the femoral canal, and inguinal lymph nodes within the femoral canal (NAVEL). The femoral canal is the site of **femoral hernias**.

Clinical Correlate

Tibial shaft fractures can cause lacerations of the anterior or posterior tibial arteries, producing either anterior or posterior **compartment syndromes.**

HIP

The hip joint is formed by the **head of the femur** and the **acetabulum.**

The fibrous capsule of the hip joint is reinforced by 3 ligamentous thickenings: **iliofemoral ligament, ischiofemoral ligament,** and **pubofemoral ligament.**

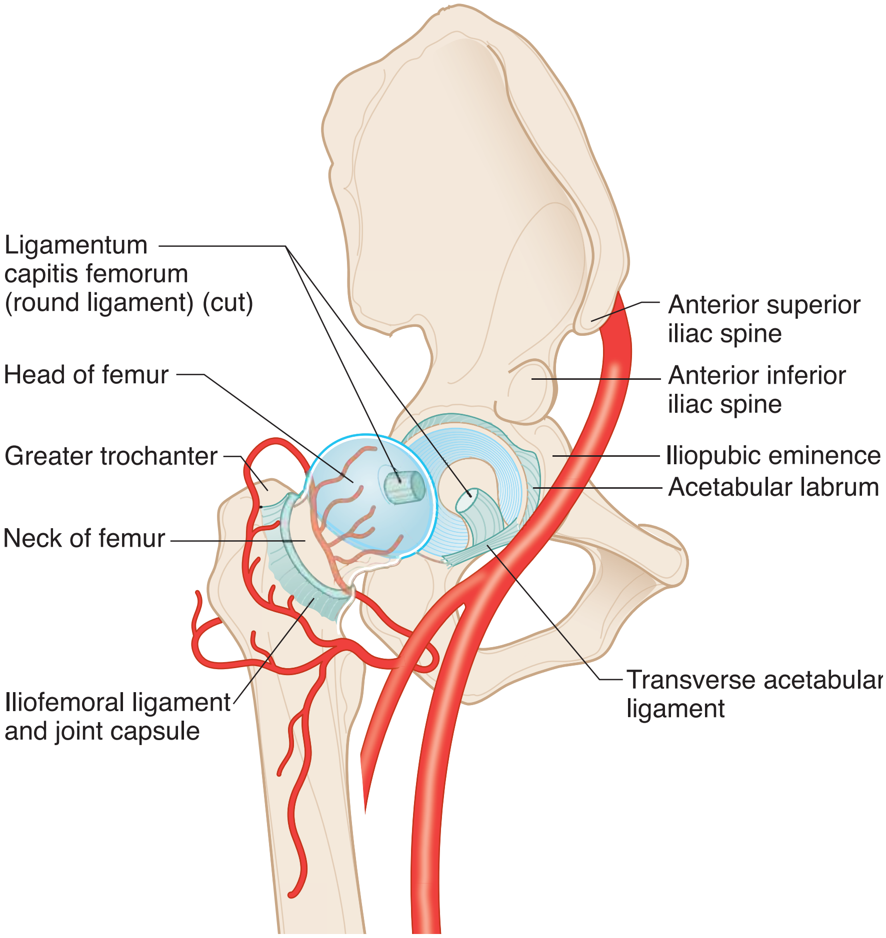

Figure II-5-4. Hip

Most of the blood supply to the head of the femur (arising mostly from the medial femoral circumflex artery) ascends along the neck of the femur. Fracture of the femoral neck can compromise this blood supply and lead to **avascular necrosis of the head of the femur.**

KNEE JOINT

The knee joint is a synovial joint formed by the articulations of the **medial and lateral femoral condyles**, the **medial and lateral tibial condyles**, and the **patella**. The primary movement at the knee joint is flexion and extension of the leg.

The knee joint is a weight-bearing joint and its stability depends on the muscles (quadriceps and hamstring muscles) that cross the joint. The knee is strengthened by several sets of ligaments.

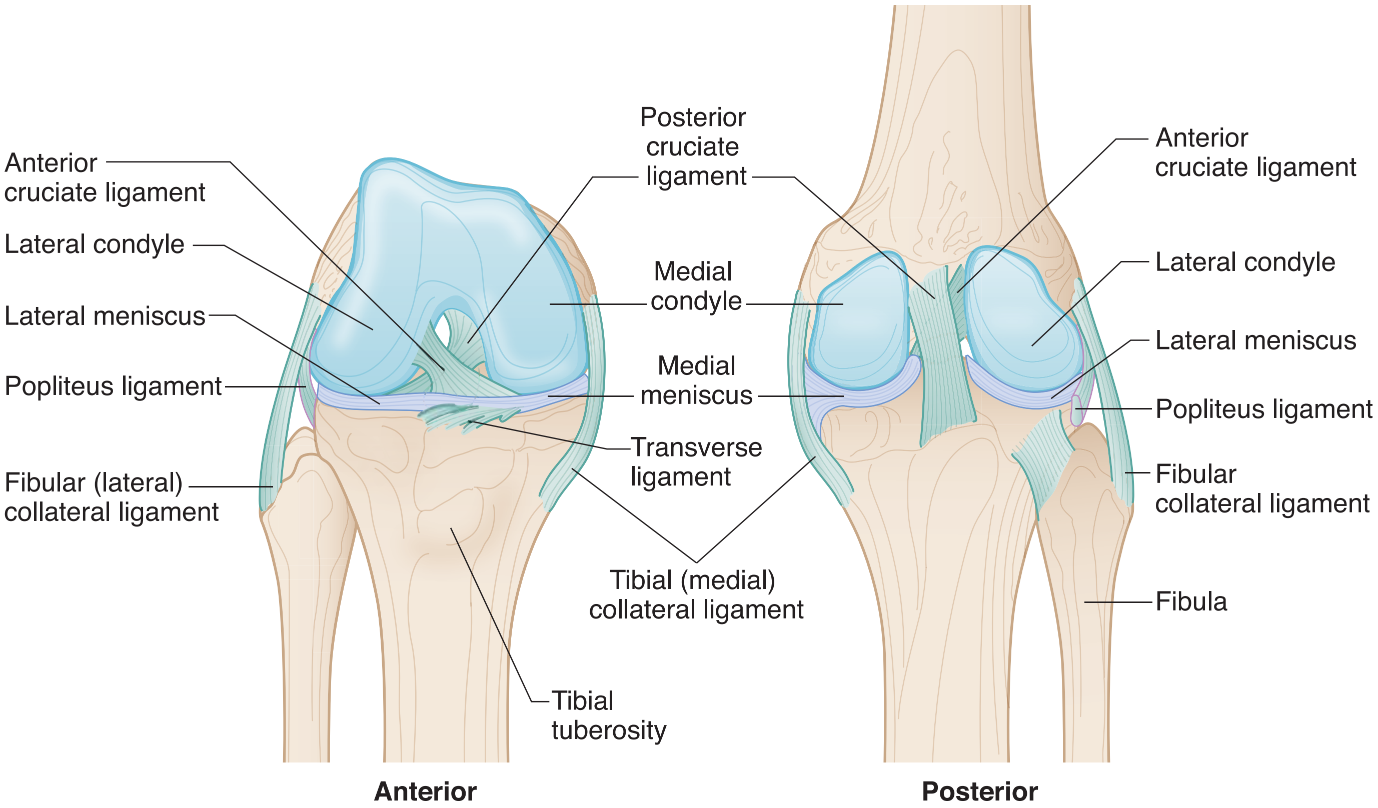

Figure II-5-5. Structures of the Knee

Tibial (Medial) and Fibular (Lateral) Collateral Ligaments

Tibial collateral ligament extends from the medial epicondyle of the femur inferiorly to attach to the medial aspect of the tibia. It is firmly attached to the capsule and medial meniscus. The tibial ligament prevents **lateral displacement** (abduction) of the tibia under the femur.

Fibular collateral ligament extends from the lateral condyle of the femur inferiorly to attach to the head of the fibula and is not attached to the lateral meniscus. The fibular ligament prevents **medial displacement** (adduction) of the tibia under the femur.

The collateral ligaments are taut with knee extension.

Clinical Correlate

The tibial collateral ligament is the most frequently torn ligament at the knee, commonly seen following lateral trauma to the knee.

Clinical Correlate

The tests for the integrity of the anterior and posterior cruciate ligaments are the **anterior and posterior drawer signs.** Tearing of the anterior cruciate ligaments allows the tibia to be easily pulled **forward** (anterior drawer sign). Tearing of the posterior cruciate ligament allows the tibial to be easily pulled **posteriorly** (posterior drawer sign).

Anterior and Posterior Cruciate Ligaments

These are intracapsular ligaments but are located outside the synovial membrane.

- **Anterior cruciate ligament** (ACL) attaches to the anterior aspect of the tibia and courses superiorly, posteriorly, and laterally to attach to the lateral condyle of the femur. The anterior ligament prevents **anterior displacement** of the tibia under the femur. Tension on the ACL is greatest when the knee is extended and resists hyperextension. It is weaker than the posterior cruciate ligament.
- **Posterior cruciate ligament** (PCL) attaches to the posterior aspect of the tibia and courses superiorly, anteriorly, and medially to attach to the medial condyle of the femur. The PCL prevents **posterior displacement** of the tibia under the femur. Tension on the PCL is greatest when the knee is flexed.

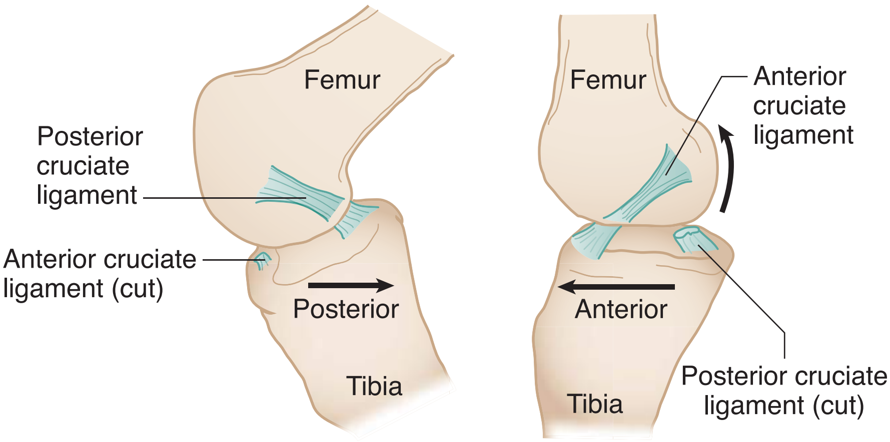

Figure II-5-6. Anterior and Posterior Cruciate Ligaments

Medial and Lateral Menisci

These are intracapsular wedges of fibrocartilage located between the articulating condyles that help make the articulating surfaces more congruent and also serve as shock absorbers.

- **Medial meniscus** is C-shaped and is firmly attached to the tibial collateral ligament. Therefore, it is less mobile and is more frequently injured than the lateral meniscus.
- **Lateral meniscus** is circular and more mobile. It is not attached to the fibular collateral ligament.

Common Knee Injuries

The 3 **most commonly injured structures at the knee** are the tibial collateral ligament, the medial meniscus, and the ACL (the terrible or unhappy triad)—injury usually results from a blow to the lateral aspect of the knee with the foot on the ground.

Patients with a **medial meniscus tear** have pain when the leg is medially rotated at the knee.

ANKLE JOINT

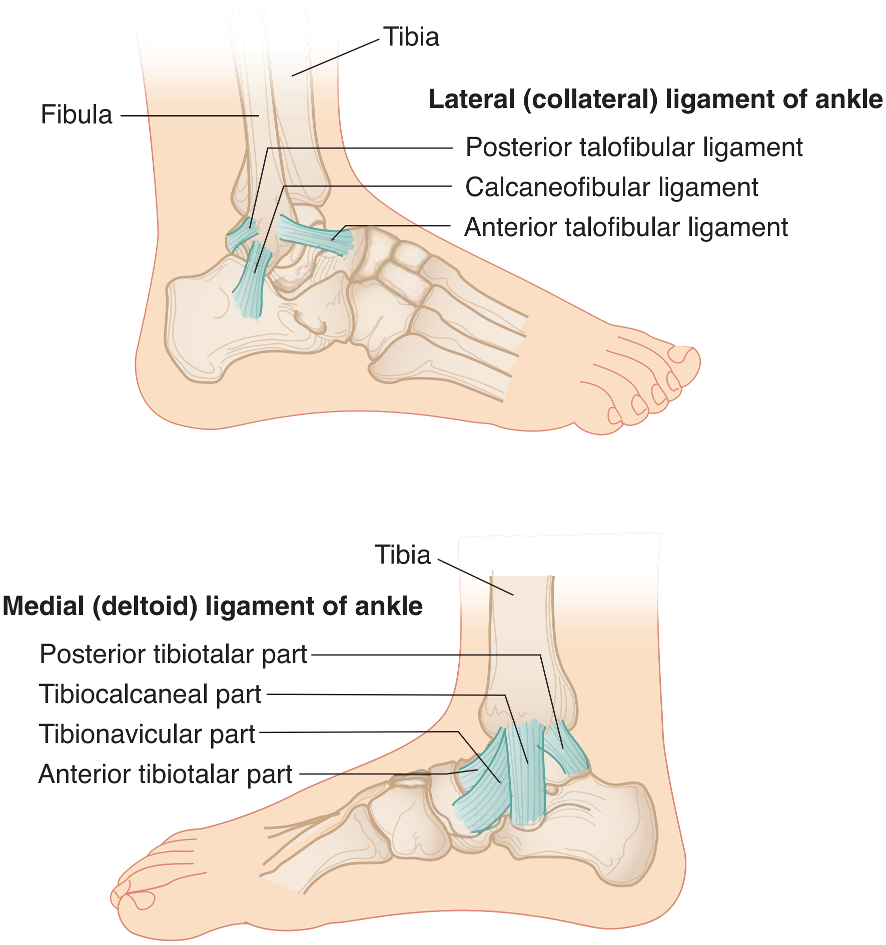

Figure II-5-7. Structures of the Ankle

Clinical Correlate

- Inversion sprains are most common.
- Anterior talofibular ligament is frequently damaged.

RADIOLOGY

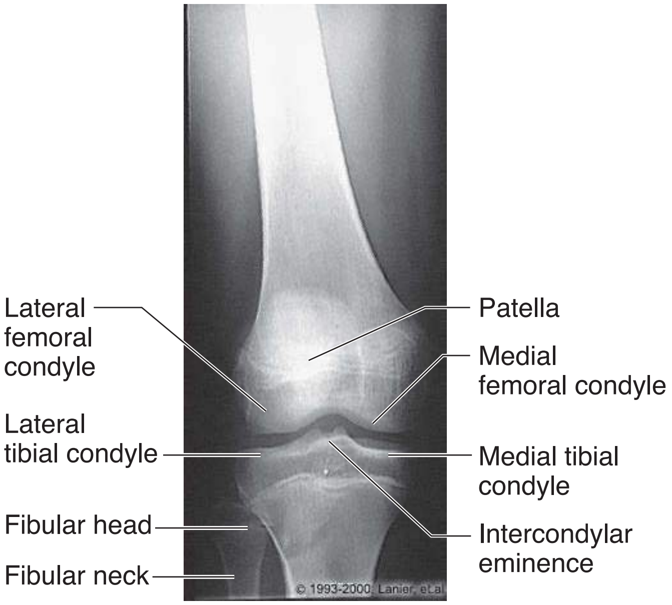

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Figure II-5-8. Lower Extremities: Anteroposterior View of Knee

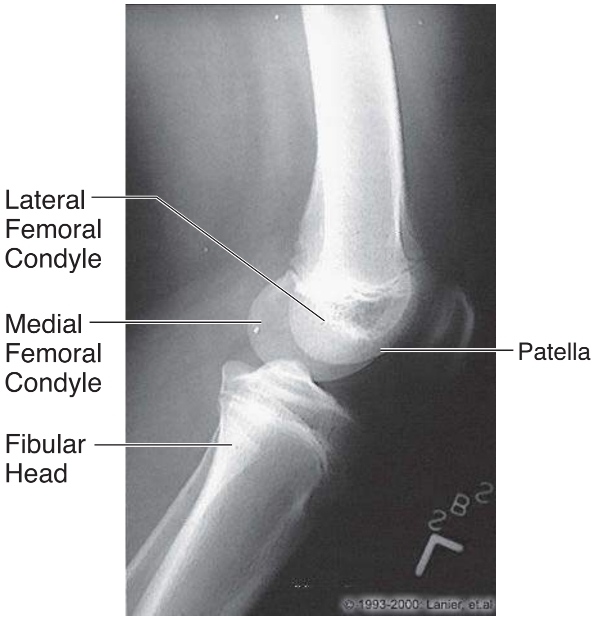

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Figure II-5-9. Lower Extremities: Lateral Knee

Head and Neck 6

Learning Objectives

- ❏ Explain information related to neck
- ❏ Answer questions about carotid and subclavian arteries
- ❏ Demonstrate understanding of embryology of the head and neck
- ❏ Solve problems concerning cranium
- ❏ Answer questions about cranial meninges and dural venous sinuses
- ❏ Use knowledge of intracranial hemorrhage
- ❏ Interpret scenarios on orbital muscles and their innervation

NECK

The **thoracic outlet** is the space bounded by the manubrium, the first rib, and T1 vertebra. The interval between the anterior and middle scalene muscles and the first rib **(scalene triangle)** transmits the structures coursing between the thorax, upper limb and lower neck. The triangle contains the **trunks of the brachial plexus** and the **subclavian artery**.

Thoracic outlet syndrome results from the compression of the **trunks of the brachial plexus and the subclavian artery** within the scalene triangle. Compression of these structures can result from tumors of the neck (Pancoast on apex of lung), a cervical rib or hypertrophy of the scalene muscles. The lower trunk of the brachial plexus (C8, T1) is usually the first to be affected. Clinical symptoms include the following:

- Numbness and pain on medial aspect of the forearm and hand
- Weakness of the muscles supplied by ulnar nerve in the hand (claw hand)
- Decreased blood flow into upper limb, indicated by weakened radial pulse

Compression can also affect the cervical sympathetic trunk (Horner's syndrome) and the recurrent laryngeal nerves (hoarseness).

Note

The subclavian vein and phrenic nerve (C 3, 4, and 5) are on the anterior surface of the anterior scalene muscle and are **not** in the scalene triangle.

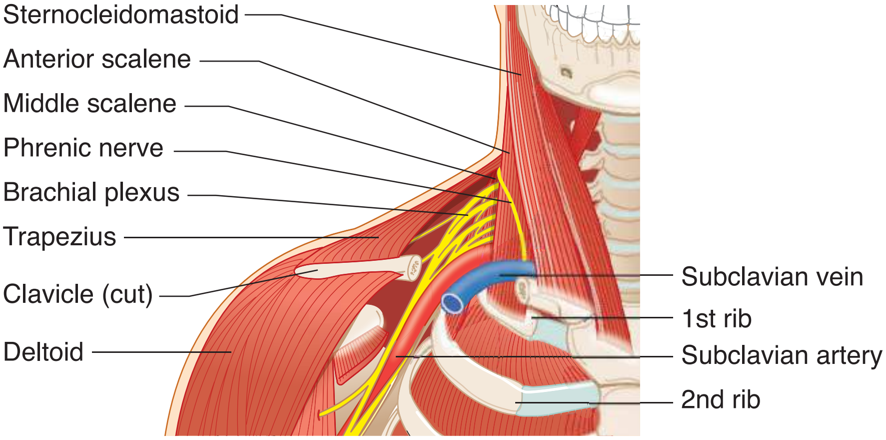
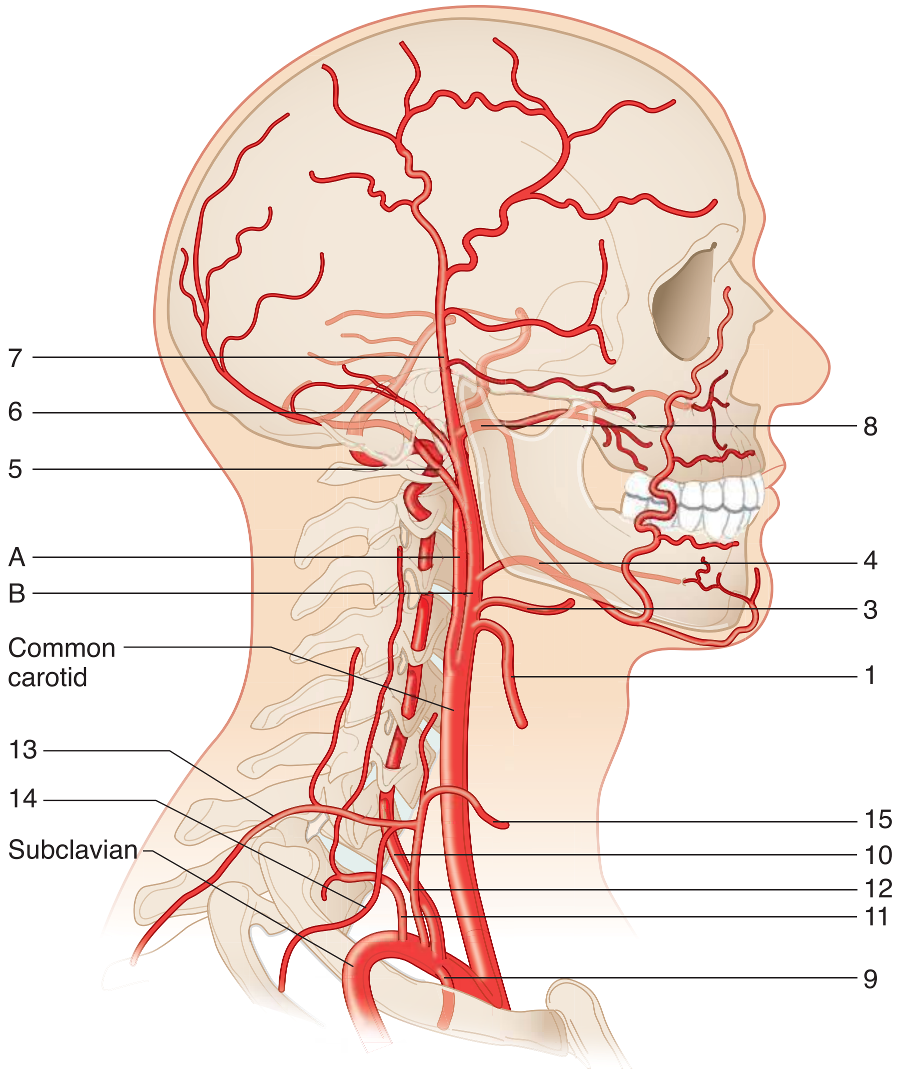


Figure II-6-1. Scalene Triangle of the Neck

CAROTID AND SUBCLAVIAN ARTERIES



Common Carotid Artery
A. Internal carotid artery—ophthalmic artery and brain
B. External carotid artery
1. Superior thyroid
2. Ascending pharyngeal (not shown)
3. Lingual
4. Facial
5. Occipital
6. Posterior auricular
7. Superficial temporal
8. Maxillary—deep face; middle meningeal artery
Subclavian Artery
9. Internal thoracic—cardiac bypass
10. Vertebral—brain
11. Costocervical
12. Thyrocervical
13. Transverse cervical
14. Suprascapular—collaterals to shoulder
15. Inferior thyroid

Figure II-6-2. Arteries to the Head and Neck

Clinical Correlate

The most significant artery of the external carotid system is the middle meningeal artery. It arises from the maxillary artery in the infratemporal fossa and enters the skull through the foramen spinosum to supply skull and dura. Lacerations of this vessel result in an epidural hematoma.

EMBRYOLOGY OF THE HEAD AND NECK

Pharyngeal Apparatus

The pharyngeal apparatus consists of the following:

- **Pharyngeal arches** (1, 2, 3, 4, and 6) composed of **mesoderm and neural crest**
- **Pharyngeal pouches** (1, 2, 3, 4) lined with **endoderm**
- **Pharyngeal grooves or clefts** (1, 2, 3, and 4) lined **with ectoderm**

The anatomic associations relating to these structures in the fetus and adult are summarized below.

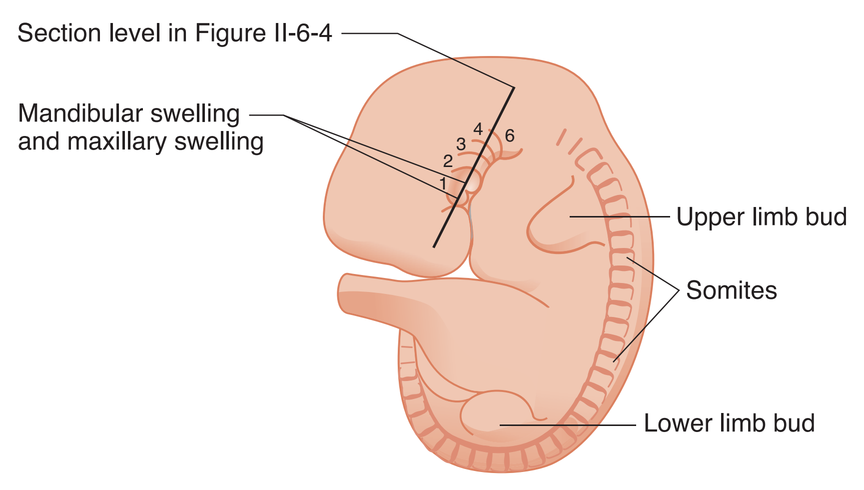


Figure II-6-3. Fetal Pharyngeal Apparatus

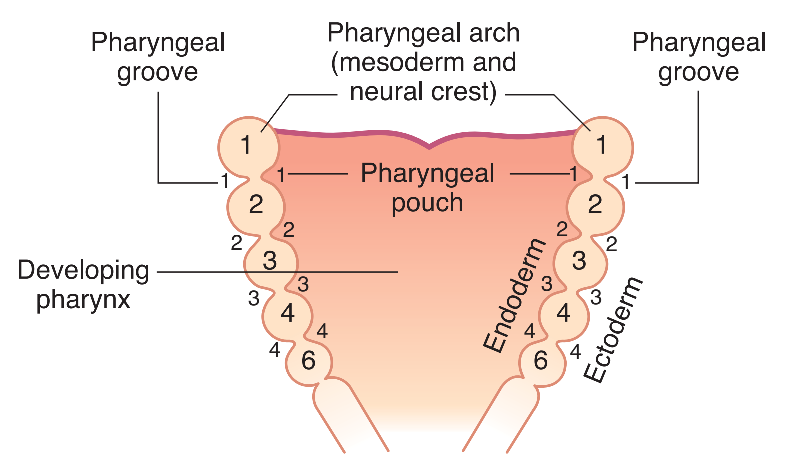


Figure II-6-4. Section through the Developing Pharynx

The components of the **pharyngeal arches** are summarized below.

Table II-6-1. Components of the Pharyngeal Arches

Arch	Nerve* (Neural Ectoderm)	Artery (Aortic Arch Mesoderm)	Muscle (Mesoderm)	Skeletal/Cartilage (Neural Crest)
1	Trigeminal: mandibular nerve		Four muscles of mastication: • Masseter • Temporalis • Lateral pterygoid • Medial pterygoid Plus: • Digastric (anterior belly) • Mylohyoid • Tensor tympani • Tensor veli palatini	Maxilla Mandible Incus Malleus
2	VII		Muscles of facial expression: Plus: • Digastric (posterior belly) • Stylohyoid • Stapedius	Stapes Styloid process Lesser horn and upper body of hyoid bone
3	IX	Right and left common carotid arteries Right and left internal carotid arteries	Stylopharyngeus muscle	Greater horn and lower body of hyoid bone
4	X – Superior laryngeal nerve – Pharyngeal branches	Right subclavian artery (right arch) Arch of aorta (left arch)	Cricothyroid muscle Soft palate Pharynx (5 muscles)	Thyroid cartilage
6	X Recurrent laryngeal nerve	Right and left pulmonary arteries Ductus arteriosus (left arch)	Intrinsic muscles of larynx (except cricothyroid muscle)	All other laryngeal cartilages

*Nerves are not derived from pharyngeal arch; they grow into the arch.

Note: The **ocular muscles** (III, IV, VI) and the **tongue muscles** (XII) do not derive from pharyngeal arch mesoderm but from mesoderm of the occipital somites (somitomeres).

The anatomic structures relating to the **pharyngeal pouches** are summarized below.

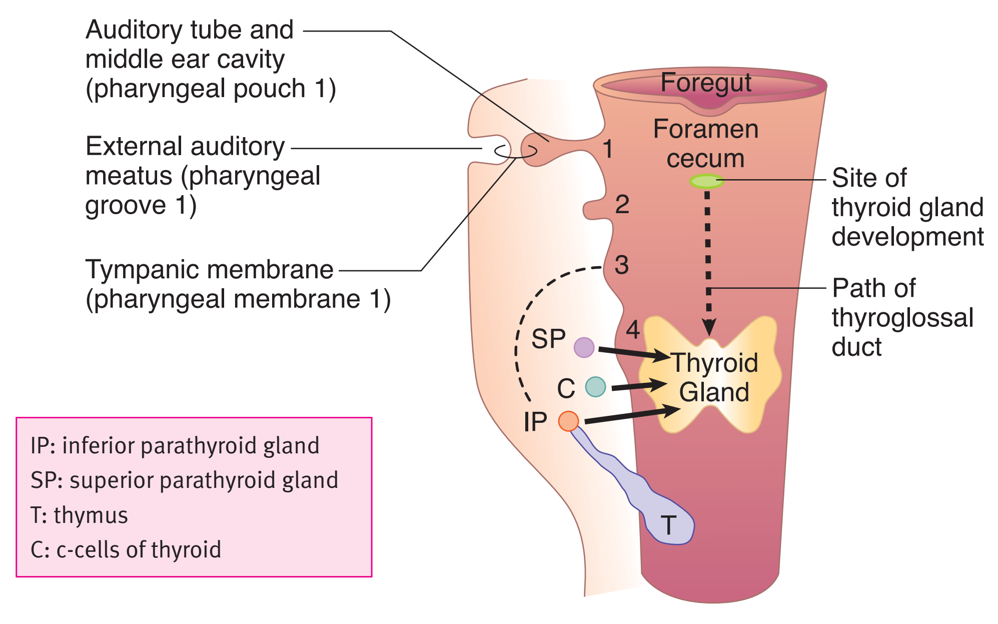


Figure II-6-5. Fetal Pharyngeal Pouches

The adult structures derived from the fetal pharyngeal pouches are summarized below.

Clinical Correlate

Normally, the second, third, and fourth pharyngeal grooves are obliterated by overgrowth of the second pharyngeal arch. Failure of a cleft to be completely obliterated results in a branchial cyst or **lateral cervical cyst .**

Clinical Correlate

The **DiGeorge sequence** presents with immunologic problems and hypocalcemia, and may be combined with cardiovascular defects (persistent truncus arteriosus), abnormal ears, and micrognathia.

Table II-6-2. Adult Structures Derived From the Fetal Pharyngeal Pouches

Pouch	Adult Derivatives
1	Epithelial lining of auditory tube and middle ear cavity
2	Epithelial lining of crypts of palatine tonsil
3	Inferior parathyroid (IP) gland Thymus (T)
4	Superior parathyroid (SP) gland C-cells of thyroid

*Neural crest cells migrate to form parafollicular C-cells of the thyroid.

Pharyngeal groove 1 gives rise to the epithelial lining of **external auditory meatus.** All other grooves are obliterated.

Thyroid Gland

The thyroid gland does not develop from a pharyngeal pouch. It develops from the **thyroid diverticulum,** which forms from the midline endoderm in the floor of the pharynx.

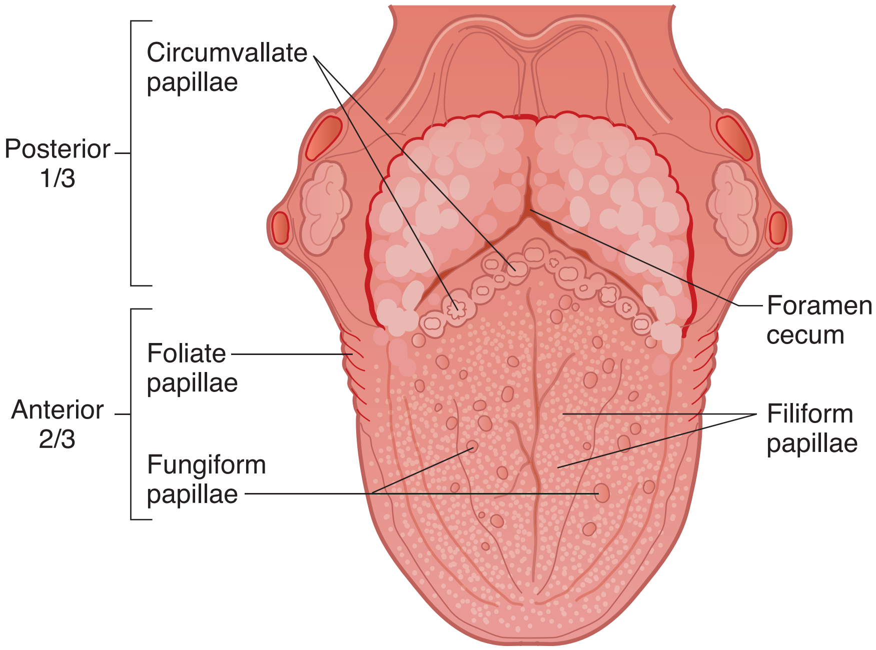
- The thyroid diverticulum migrates caudally to its adult anatomic position in the neck but remains connected to the foregut via the **thyroglossal duct,** which is later obliterated.
- The former site of the thyroglossal duct is indicated in the adult by the **foramen cecum**.

Tongue

The **anterior two-thirds of the tongue** is associated with **pharyngeal arches 1 and 2.** General sensation is carried by the lingual branch of the mandibular nerve (cranial nerve [CN] V). Taste sensation is carried by **chorda tympani of CN VII**.

The **posterior one third of the tongue** is associated with **pharyngeal arch 3.** General sensation and taste are carried by **CN IX.**

Most of the muscles of the tongue are innervated by CN XII.



Sensory		
	General sensation	*Taste*
Post 1/3	IX	IX
Ant 2/3	V	VII
	Lingual branch of mandibular	Chorda tympani branch of VII nerve

Somatic Motor
CN XII innervates the intrinsic and extrinsic skeletal muscles of the tongue except palatoglossus muscle.

Figure II-6-6. Tongue

Clinical Correlate

Cleft lip occurs when the maxillary prominence fails to fuse with the medial nasal prominence.

Cleft palate occurs when the palatine shelves fail to fuse with each other or the primary palate.

Face and Palate

The **face** develops from 5 primordia of mesoderm (neural crest) of the first pharyngeal arch: a single frontonasal prominence, the pair of maxillary prominences, and the pair of mandibular prominences.

- The **intermaxillary segment** forms when the 2 medial nasal prominences of the frontonasal prominences fuse together at the midline and form the **philtrum of the lip** and the **primary palate**.
- The **secondary palate** forms from palatine shelves (maxillary prominence), which fuse in the midline, posterior to the incisive foramen.
- The primary and secondary palates fuse at the **incisive foramen** to form the definitive hard palate.

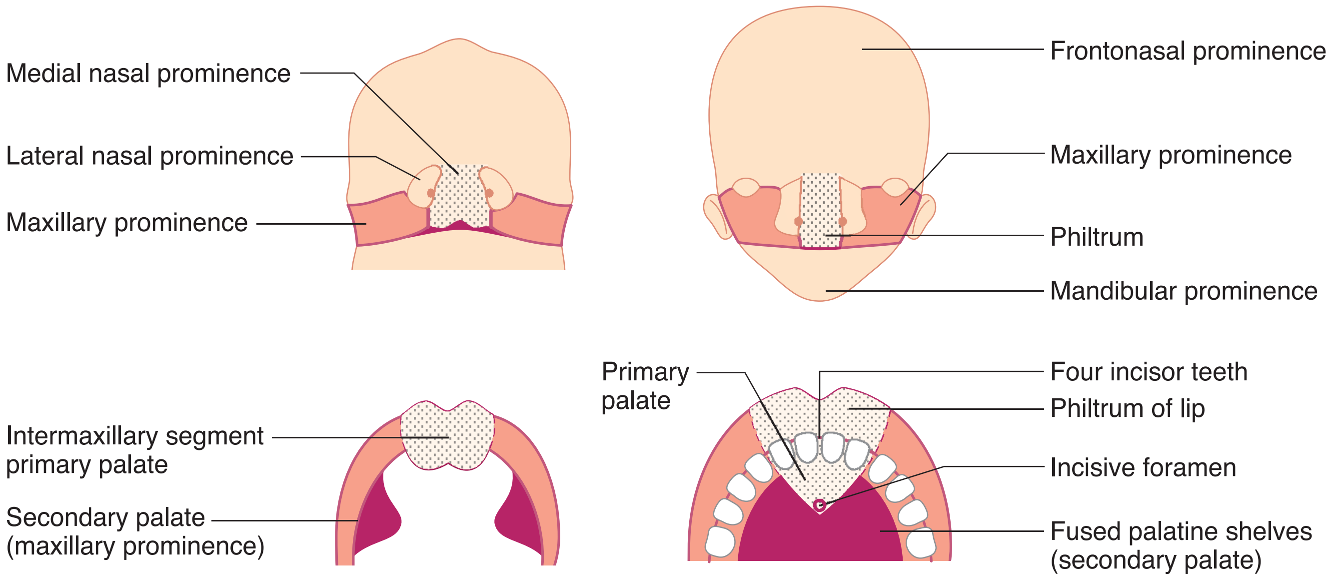


Figure II-6-7. Face and Palate Development

Clinical Correlate

First arch syndrome results from abnormal formation of pharyngeal arch 1 because of faulty migration of neural crest cells, causing facial anomalies. Two well-described syndromes are Treacher Collins syndrome and Pierre Robin sequence. Both defects involve neural crest cells.

Pharyngeal fistula occurs when pouch 2 and groove 2 persist, thereby forming a fistula generally found along the anterior border of the muscle.

Pharyngeal cyst occurs when pharyngeal grooves that are normally obliterated persist, forming a cyst usually located at the angle of the mandible.

Ectopic thyroid, parathyroid, or thymus results from abnormal migration of these glands from their embryonic position to their adult anatomic position. Ectopic thyroid tissue is found along the midline of the neck. Ectopic parathyroid or thymus tissue is generally found along the lateral aspect of the neck. May be an important issue during neck surgery.

Thyroglossal duct cyst or fistula occurs when parts of the thyroglossal duct persist, generally in the midline near the hyoid bone. The cyst may also be found at the base of the tongue (lingual cyst).

DiGeorge sequence occurs when pharyngeal pouches 3 and 4 fail to differentiate into the parathyroid glands and thymus. Neural crest cells are involved.

Clinical Correlate

Robin sequence presents with a triad of poor mandibular growth, cleft palate, and a posteriorly placed tongue.

Treacher Collins syndrome also presents with mandibular hypoplasia, zygomatic hypoplasia, down-slanted palpebral fissures, colobomas, and malformed ears.

Clinical Correlate

Cribriform plate fractures may result in dysosmia and rhinorrhea (CSF).

CRANIUM

Cranial Fossae

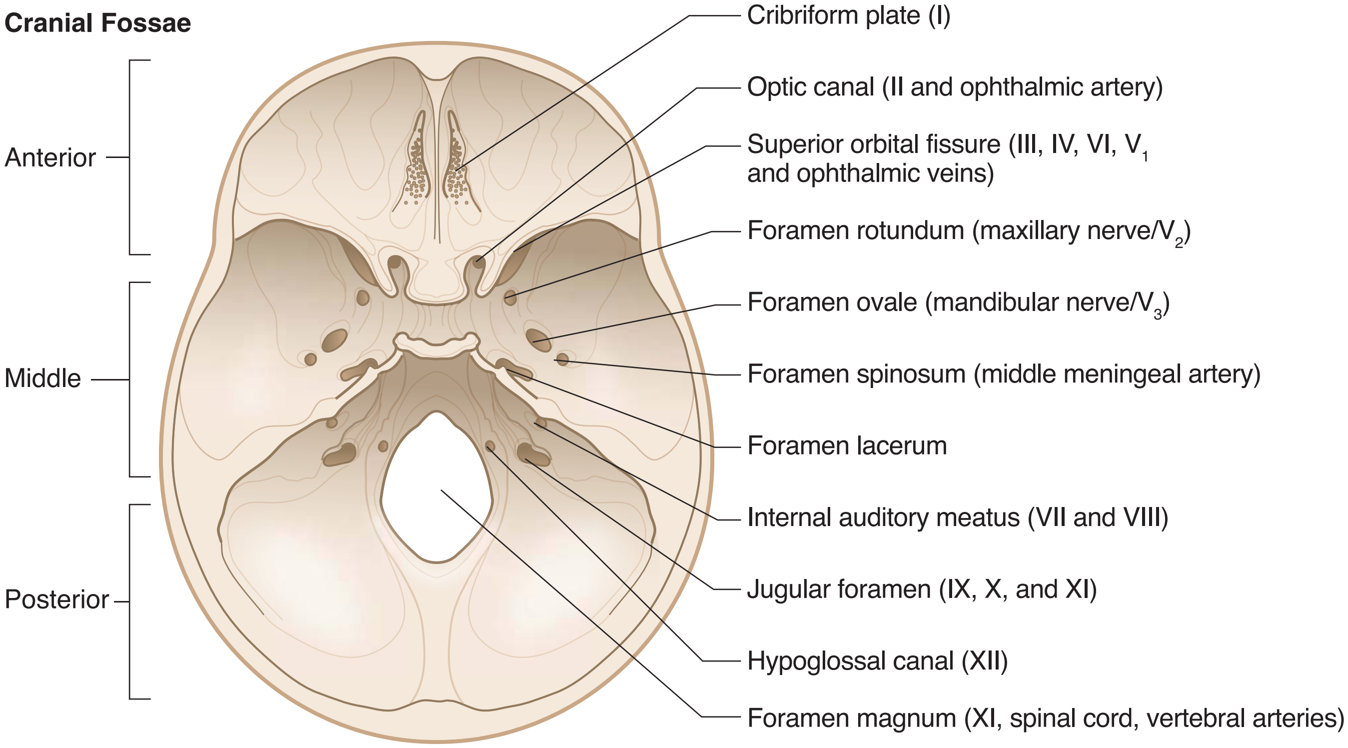


Figure II-6-8. Foramina: Cranial Fossae

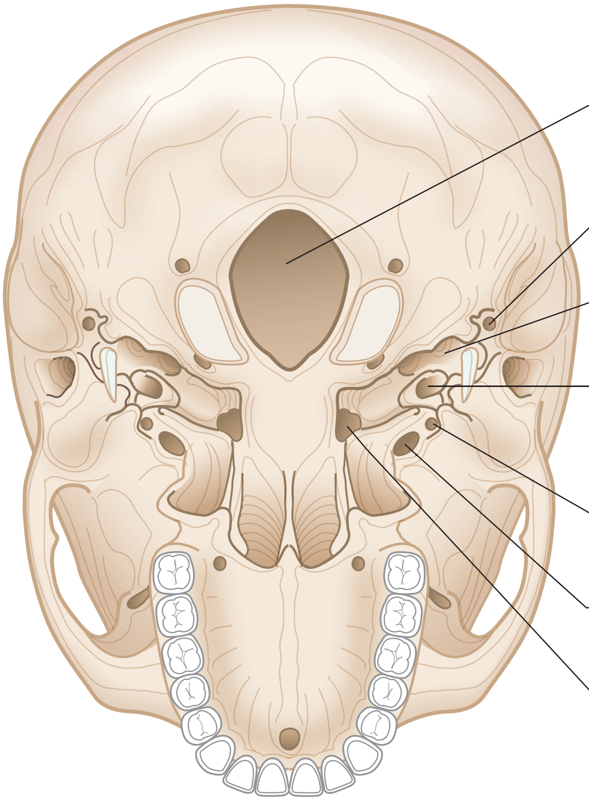


Figure II-6-9. Foramina: Base of Skull

Clinical Correlate

Jugular foramen syndrome may be caused by a tumor pressing on CN IX, X, and XI. Patients present with hoarseness, dysphagia (CN IX and X), loss of sensation over the oropharynx and posterior third of the tongue (CN IX), and trapezius and sternocleidomastoid weakness (CN XI). The nearby CN XII may be involved, producing tongue deviation to the lesioned side.

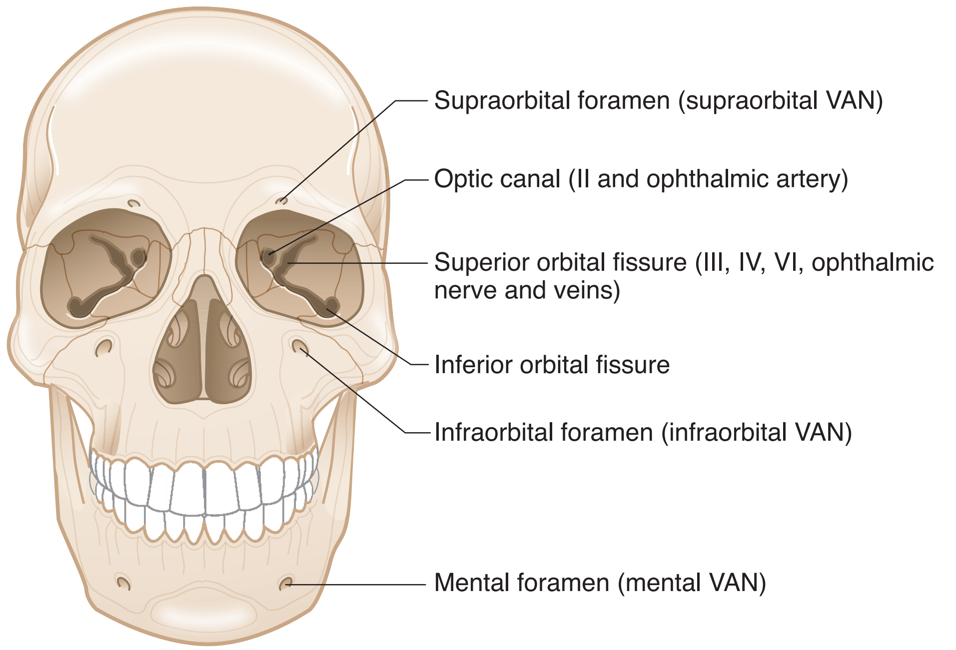


Figure II-6-10. Foramina: Front of Skull

CRANIAL MENINGES AND DURAL VENOUS SINUSES

Cranial Meninges

The brain is covered by 3 meninges that are continuous through the foramen magnum with the spinal meninges. There are several similarities and differences between spinal and cranial meninges.

- **Pia mater** tightly invests the surfaces of the brain and cannot be dissected away, having the same relationship with the brain as spinal pia mater.
- **Dura mater** (thickest) unlike the spinal dura, consists of 2 layers (**periosteal and meningeal**) that are fused together during most of their course in the cranial cavity.
  - **Periosteal layer**: outer layer lines the inner surfaces of the flat bones and serves as their periosteum; can easily be peeled away from the bones
  - **Meningeal (true dura) layer**: innermost layer that is mostly fused with the periosteal dura mater throughout the cranial cavity. At certain points in the cranium, the meningeal layer separates from the periosteal layer and forms the dural venous sinuses and connective tissue foldings or duplications: **falx cerebri, diaphragma sellae and tentorium cerebelli**. These duplications separate and support different parts of the CNS.

- **Arachnoid** is the thin, delicate membrane which lines and follows the inner surface of the meningeal dura. Projections of arachnoid called **arachnoid granulations** penetrate through the dura mater and extend into the superior sagittal dural venous sinus. Arachnoid granulations are where CSF returns to the systemic venous circulation.

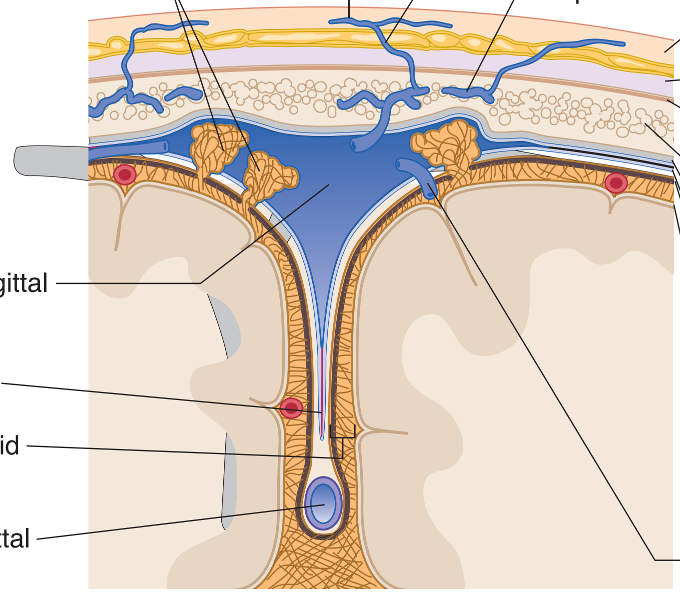


Figure II-6-11. Coronal Section of the Dural Sinuses

There are several spaces related to the cranial meninges:

- **Epidural space** is a potential space between the periosteal dura and the bones of the skull: site of epidural hematomas (described later).
- **Subdural space** is the potential space between the meningeal dura and the arachnoid membrane: site of subdural hematomas (described later).
- **Subarachnoid space** lies between the arachnoid and pia mater containing CSF: site of subarachnoid hemorrhage (described later).

Dural Venous Sinuses

Dural venous sinuses are formed at different points in the cranial cavity where the **periosteal** and **meningeal** dural layers separate to form endothelial lined venous channels called dural venous sinuses (Figures II-6-11 and II-6-12). The sinuses provide the major venous drainage from most structures within the cranial cavity. They drain mostly into the internal jugular vein, which exits the cranial floor at the jugular foramen. Most of the dural venous sinuses are located in the 2 largest duplications of meningeal dura mater (**falx cerebri** and the **tentorium cerebelli**).

The primary tributaries that flow into the sinuses are the following:

- **Cerebral** and **cerebelli veins** form **bridging veins,** which pass across the subdural space to drain into the sinuses.
- **Emissary** veins are valveless channels that course through the bones of the skull and allow dural sinuses to communicate with extracranial veins.
- **Diploic veins** drain the spongy (diploe) core of the flat bones.
- **Arachnoid granulations** are where CSF returns to the venous circulation.
- **Meningeal veins** drain the meninges.

Names of the Major Dural Sinuses

1. Superior sagittal*
2. Inferior sagittal
3. Straight*
4. Transverse* (2)
5. Sigmoid (2)
6. Cavernous (2)
7. Superior petrosal (2)

* Drain into the confluence of sinuses located at the inion.

Folds (Duplications) of Dura Mater

A. Falx cerebri
B. Tentorium cerebelli

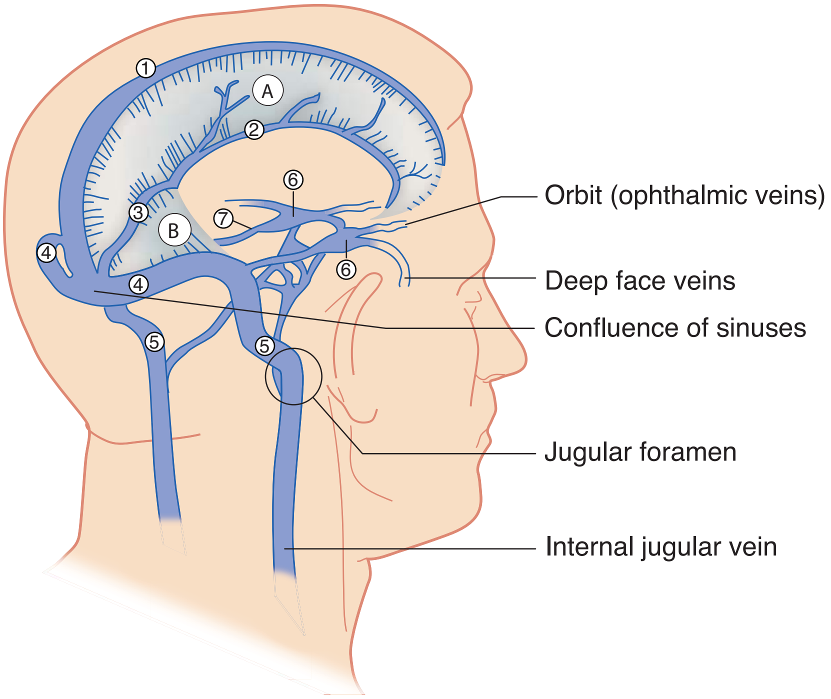


Figure II-6-12. Dural Venous Sinuses

Major dural venous sinuses

The major dural venous sinuses are the following:

- The **superior sagittal sinus** is located in the midsagittal plane along the superior aspect of the falx cerebri. It drains primarily into the confluence of the sinuses.
- The **inferior sagittal sinus** is located in the midsagittal plane near the inferior margin of the falx cerebri. It terminates by joining with the great cerebral vein (of Galen) to form the straight sinus at the junction of the falx cerebri and tentorium cerebelli.

- The **straight sinus** is formed by the union of the inferior sagittal sinus and the great cerebral vein. It usually terminates by draining into the confluence of sinuses (or into the transverse sinus).
- The **occipital sinus** is a small sinus found in the posterior border of the tentorium cerebelli. It drains into the confluence of sinuses.
- The **confluence of sinuses** is formed by the union of the superior sagittal, straight, and occipital sinuses posteriorly at the occipital bone. It drains laterally into the 2 transverse sinuses.
- The **transverse sinuses** are paired sinuses in the tentorium cerebelli and attached to the occipital bone that drain venous blood from the confluence of sinuses into the sigmoid sinuses.
- The **sigmoid sinuses** are paired and form a S-shaped channel in the floor of the posterior cranial fossa. The sigmoid sinus drains into the **internal jugular vein** at the **jugular foramen**.
- The paired **cavernous sinuses** are located on either side of the body of the sphenoid bone.
  - Each sinus receives blood primarily from the **orbit (ophthalmic veins)** and via emissary veins from the **deep face** (pterygoid venous plexus). Superficial veins of the maxillary face drain into the medial angle of the eye, enter the ophthalmic veins, and drain into the cavernous sinus.
  - Each cavernous sinus 1 via the superior and inferior petrosal sinuses into the sigmoid sinus and internal jugular vein, respectively.
  - The cavernous sinuses are the **most clinically significant** dural sinuses because of their relationship to a number of cranial nerves. **CN III** and **IV** and the **ophthalmic and maxillary** divisions of the trigeminal nerve are located in lateral wall of the sinus. **CN VI** and **internal carotid artery** are located centrally in the sinus.

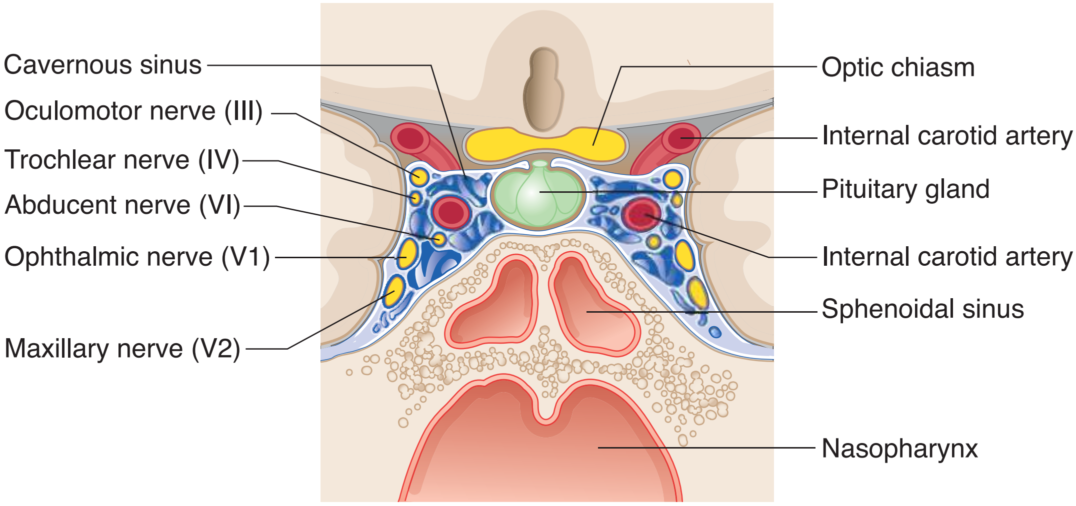


Figure II-6-13. Coronal Section Through Pituitary Gland and Cavernous Sinuses

Clinical Correlate

Cavernous Sinus Thrombosis

Infection can spread from the superficial and deep face into the cavernous sinus, producing a thrombosis that may result in swelling of sinus and damage the cranial nerves that are related to the cavernous sinus.

CN III and IV and the ophthalmic and maxillary divisions of CN V will be compressed in the lateral wall of the sinus.

CN VI and the internal carotid artery with its periarterial plexus of postganglionic sympathetic fibers will be compressed in the central part of the cavernous sinus. CN VI is typically affected first in a cavernous sinus thrombosis with the other nerves being affected later. Initially, patients have an internal strabismus (medially deviated eyeball) (CN VI lesion). Later, all eye movements are affected, along with altered sensation in the skin of the upper face and scalp.

INTRACRANIAL HEMORRHAGE

Epidural Hematoma

An **epidural hematoma** results from trauma to the lateral aspect of the skull which lacerates the middle meningeal artery. Arterial hemorrhage occurs rapidly in the epidural space between the periosteal dura and the skull.

- Epidural hemorrhage forms a **lens-shaped** (biconvex) hematoma at the lateral hemisphere.
- Epidural hematoma is associated with a momentary loss of consciousness followed by a lucid (asymptomatic) period of up to 48 hours.
- Patients then develop symptoms of elevated intracranial pressure such as headache, nausea, and vomiting, combined with neurological signs such as hemiparesis.
- Herniation of the temporal lobe, coma, and death may occur rapidly if the arterial blood is not evacuated.

Subdural Hematoma

A **subdural hematoma** results from head trauma that tears superficial ("bridging") cerebral veins at the point where they enter the superior sagittal sinus. A subdural hemorrhage occurs between the meningeal dura and the arachnoid.

- Subdural hemorrhage forms a **crescent-shaped** hematoma at the lateral hemisphere.
- Large subdural hematomas result in signs of elevated intracranial pressure such as headache and nausea.
- Small or chronic hematomas are often seen in elderly or chronic alcoholic patients.
- Over time, herniation of the temporal lobe, coma, and death may result if the venous blood is not evacuated.

Nolte: The Human Brain.
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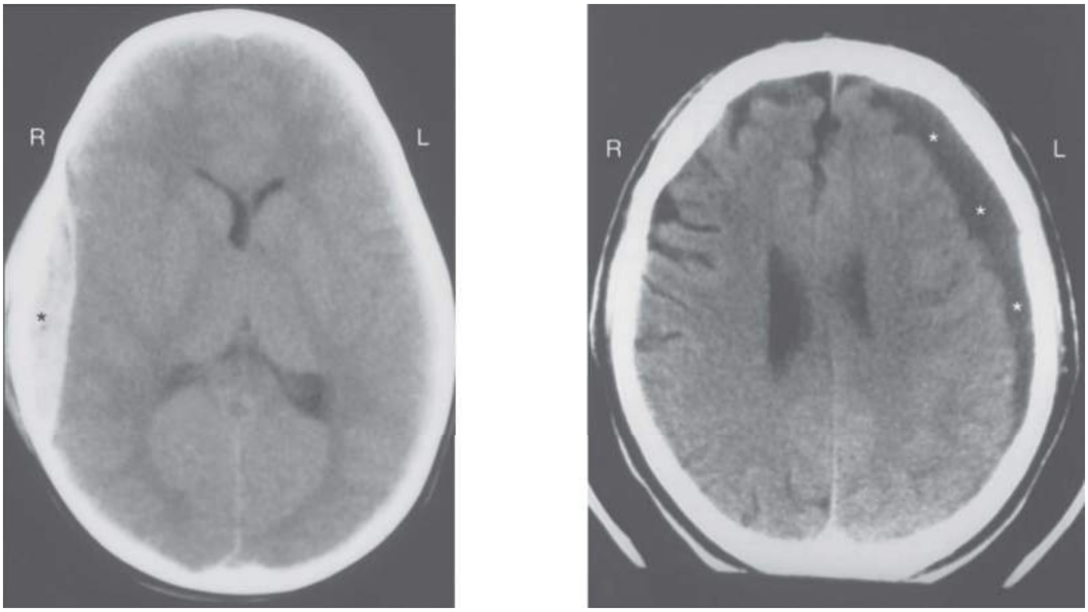

A. Epidural Hematoma* B. Subdural Hematoma*

Figure II-6-14. Intracranial Hemorrhage

Subarachnoid Hemorrhage

A **subarachnoid hemorrhage** results from a rupture of a berry aneurysm in the circle of Willis. The most common site is in the anterior part of the circle of Willis at the branch point of the anterior cerebral and anterior communicating arteries. Other common sites are in the proximal part of the middle cerebral artery or at the junction of the internal carotid and posterior communicating arteries. Typical presentation is the onset of a severe headache.

ORBITAL MUSCLES AND THEIR INNERVATION

In the orbit, there are 6 extraocular muscles that move the eyeball. A seventh muscle, the levator palpebrae superioris, elevates the upper eyelid.

- Four of the 6 extraocular muscles (the superior, inferior, and medial rectus, and the inferior oblique, plus the levator palpebrae superioris) are innervated by the oculomotor nerve (CN III).
- The superior oblique muscle is the only muscle innervated by the trochlear nerve (CN IV).
- The lateral rectus is the only muscle innervated by the abducens nerve (CN VI).
- The levator palpebrae superioris is composed of skeletal muscle innervated by the oculomotor nerve (CN III) and smooth muscle (the superior tarsal muscle) innervated by sympathetic fibers.
- Sympathetic fibers reach the orbit from a plexus on the internal carotid artery of postganglionic axons that originate from cell bodies in the superior cervical ganglion.

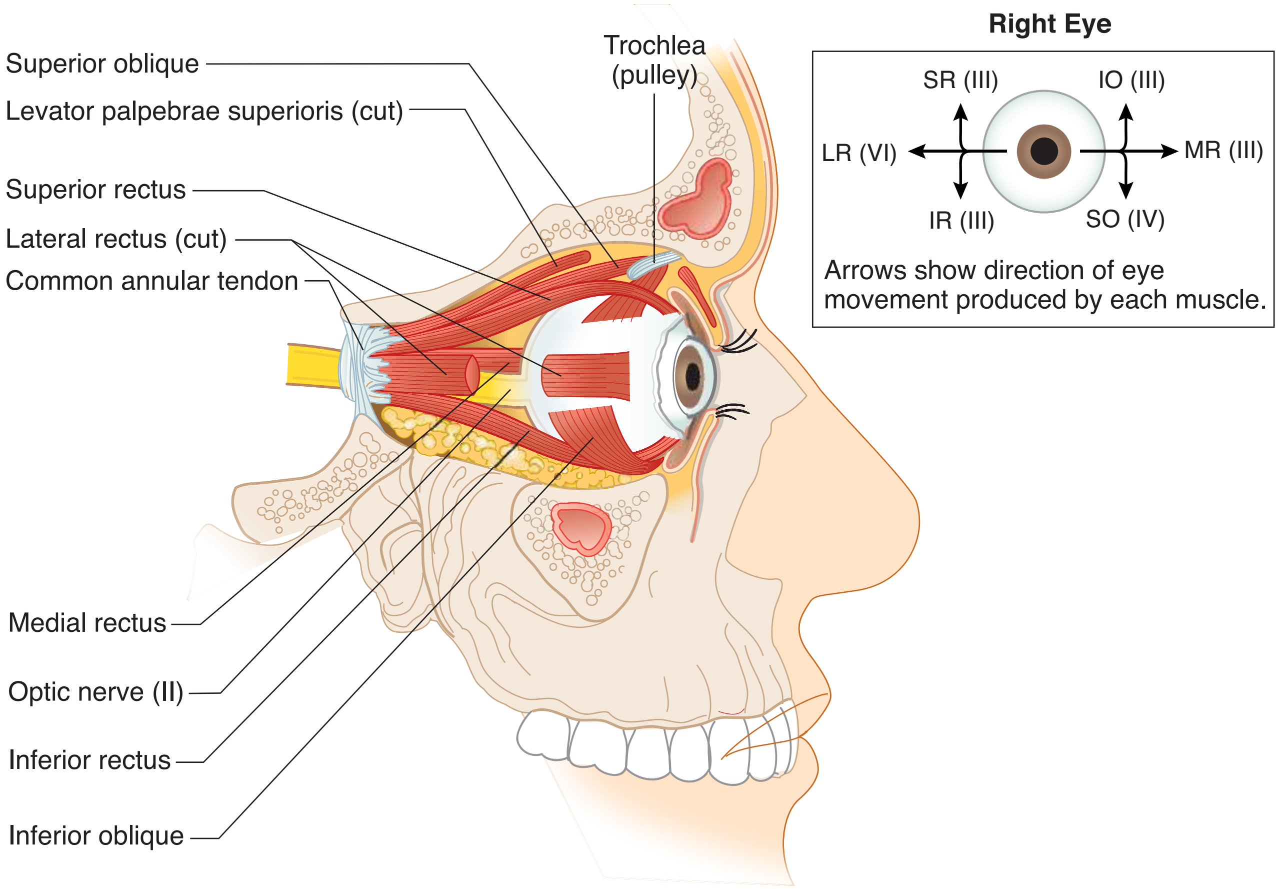

Figure II-6-15. Muscles of the Eye

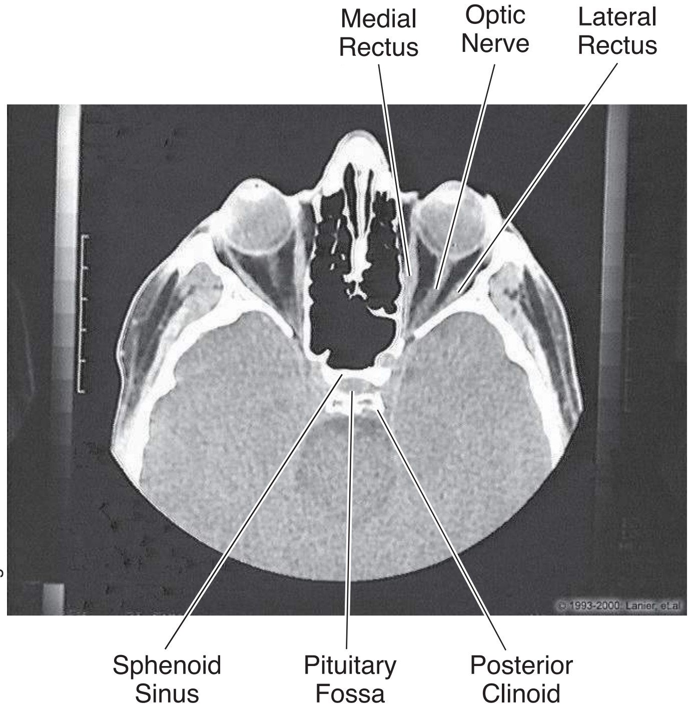

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Figure II-6-16. Head and Neck: CT, Orbit

PART III

Neuroscience

Nervous System Organization and Development

1

Learning Objectives

- ❑ Explain information related to autonomic nervous system
- ❑ Use knowledge of general organization

NERVOUS SYSTEM

The central nervous system (CNS) consists of the brain and spinal cord, which develop from the neural tube.

The peripheral nervous system (PNS) contains cranial and spinal nerves that consist of neurons that give rise to axons, which grow out of the **neural tube**, and neurons derived from **neural crest cells**. Skeletal motor neurons and axons of preganglionic autonomic neurons are derived from the neural tube.

Neural crest cells form sensory neurons and postganglionic autonomic neurons. The neuronal cell bodies of these neurons are found in ganglia. Therefore, all ganglia found in the PNS contain either sensory or postganglionic autonomic neurons and are derived from neural crest cells.

Chromaffin cells are neural crest cells, which migrate into the adrenal medulla to form postganglionic sympathetic neurons.

Development of the Nervous System

Neurulation begins in the third week; both CNS and PNS derived from neuroectoderm. The **notochord** induces the overlying ectoderm to form the **neural plate (neuroectoderm)**. By end of the third week, **neural folds** grow over midline and fuse to form **neural tube**.

- During closure, **neural crest** cells also form from neuroectoderm.
- **Neural tube** 3 primary vesicles → 5 primary vesicles → brain and spinal cord
- Brain stem and spinal cord have an **alar** plate (**sensory**) and a **basal** plate (**motor**); plates are separated by the **sulcus limitans**.
- **Neural crest** → sensory and postganglionic autonomic neurons, and other non-neuronal cell types.
- **Peripheral NS (PNS):** cranial nerves (12 pairs) and spinal nerves (31 pairs)

Note

Alpha-fetoprotein (AFP) levels may also be elevated in gastroschisis and omphalocele. AFP levels are low in pregnancy of Down syndrome fetus.

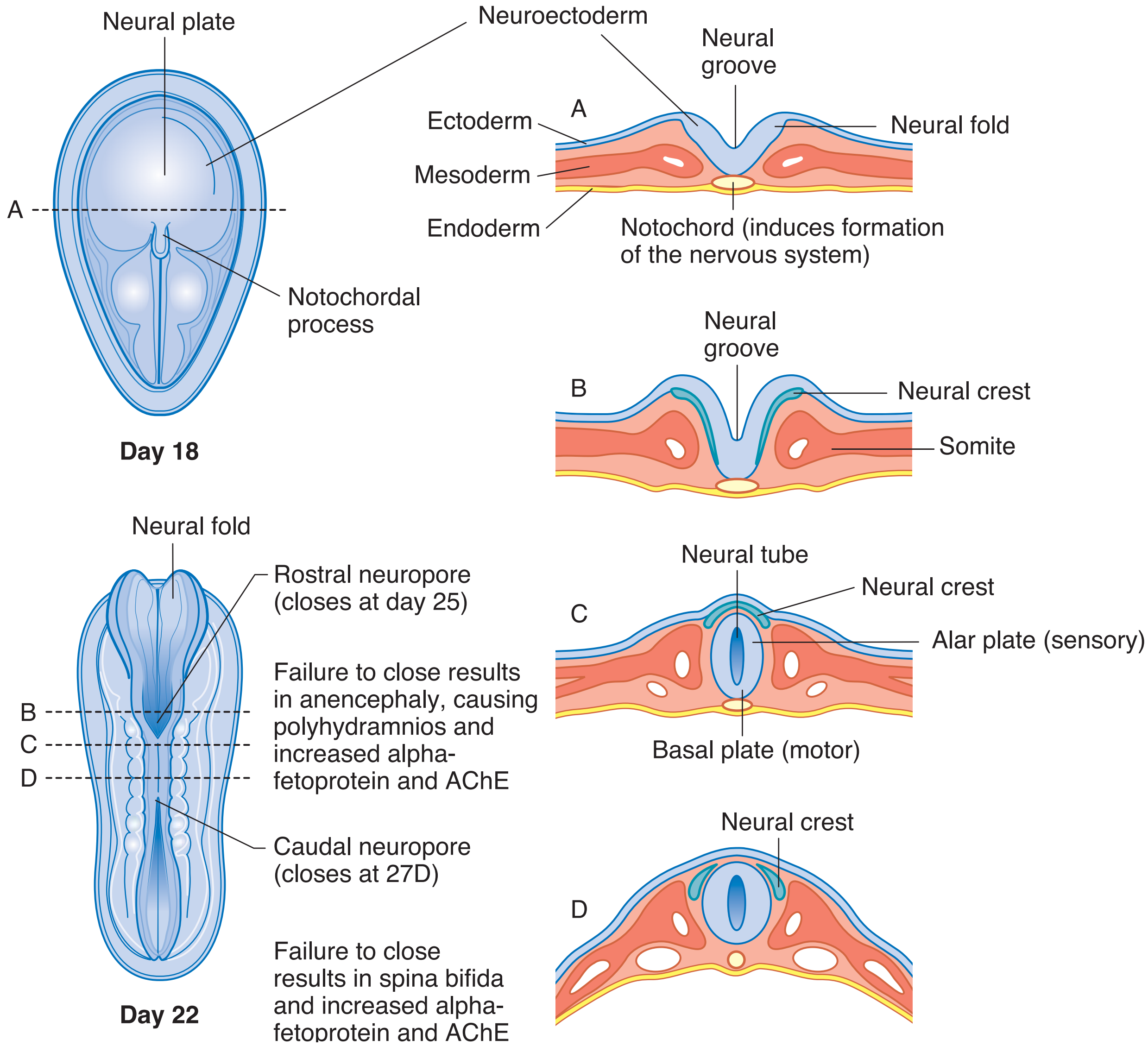


Figure III-1-1. Development of Nervous System

Central Nervous System

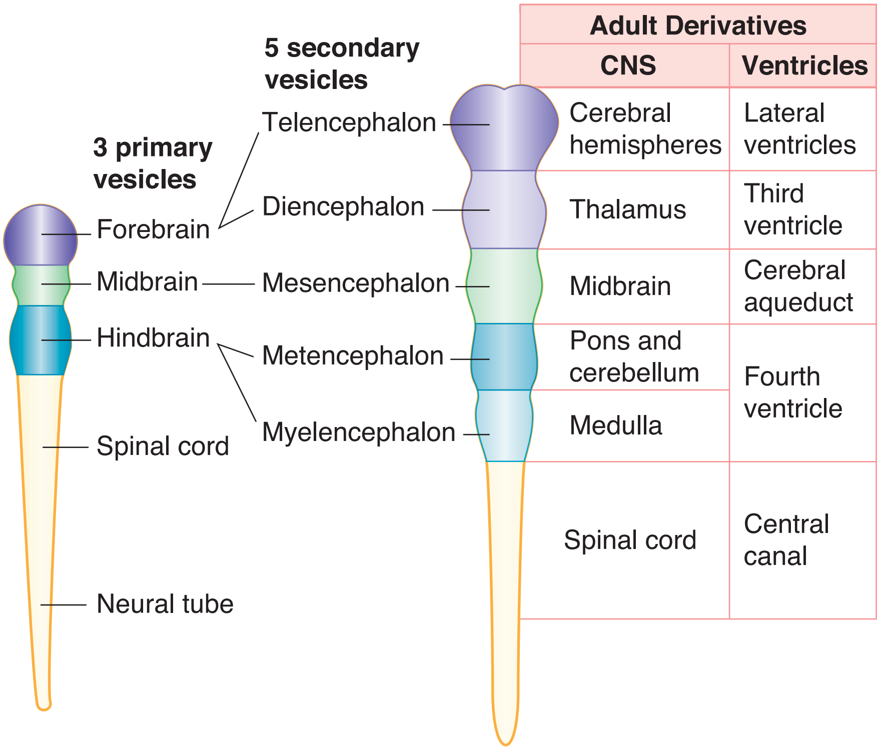


Figure III-1-2. Adult Derivatives of Secondary Brain Vesicles

Clinical Correlate

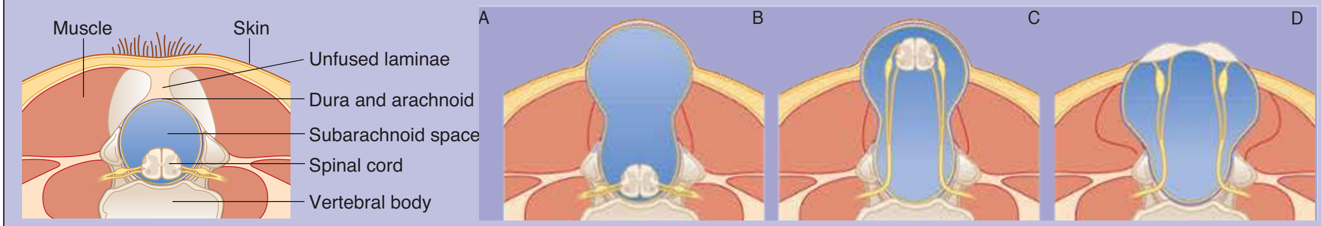
Axonal polyneuropathies produce distal "glove-and-stocking" weakness or sensory deficits, and are related to axonal transport failure. Diabetes mellitus patients present with sensory neuropathies.

Table III-1-1. Adult Derivatives of Secondary Brain Vesicles

	Structures	Neural Canal Remnant
Telencephalon	Cerebral hemispheres, most of basal ganglia	Lateral ventricles
Diencephalon	Thalamus, hypothalamus, subthalamus, epithalamus (pineal gland), retina and optic nerve	Third ventricle
Mesencephalon	Midbrain	Cerebral aqueduct
Metencephalon	Pons, cerebellum	Fourth ventricle
Myelencephalon	Medulla Spinal cord	Central canal

Table III-1-2. Congenital Malformations of the Nervous System

Condition	Types	Description
Anencephaly	—	Failure of **anterior neuropore** to close Brain does not develop Incompatible with life Increased AFP during pregnancy and AChE
Spina bifida	Spina bifida occulta **(Figure A)**	Failure to induce bone growth around the spinal cord Mildest form **Vertebrae** fail to form around spinal cord **No** increase in **AFP** Asymptomatic; tuft of hair over defect.
	Spina bifida with meningocele **(Figure B)**	**Meninges** protrude through vertebral defect Increase in AFP
	Spina bifida with meningomyelocele **(Figure C)**	**Meninges** and **spinal cord** protrude through vertebral defect; seen with Arnold-Chiari Type II Increase in AFP
	Spina bifida with myeloschisis **(Figure D)**	Most severe **Spinal cord** can be seen **externally** Increase in AFP and AChE



Arnold-Chiari malformation	Type I	Most common Mostly **asymptomatic** in children Downward displacement of cerebellar tonsils through foramen magnum Frequent association with **syringomyelia**
	Type II	More often **symptomatic** Downward displacement of **cerebellar vermis** Compression of IV ventricle → obstructive **hydrocephaly** Frequent lumbar **meningomyelocele**
Dandy-Walker malformation		Failure of foramina of Luschka and Magendie to open → **dilation of IV ventricle** Agenesis of cerebellar vermis and splenium of the corpus callosum
Hydrocephalus		Most often caused by stenosis of cerebral aqueduct CSF accumulates in ventricles and subarachnoid space Increased head circumference
Holoprosencephaly		Incomplete separation of cerebral hemispheres One ventricle in telencephalon Seen in trisomy 13 (Patau)

Abbreviation: AFP, alpha-fetoprotein

Table III-1-3. Germ Layer Derivatives

Ectoderm	Mesoderm	Endoderm
Surface ectoderm Epidermis Hair Nails Inner ear, external ear Enamel of teeth Lens of eye Anterior pituitary (Rathke's pouch) Parotid gland **Neuroectoderm** **Neural tube** Central nervous system Retina and optic nerve Pineal gland Neurohypophysis Astrocytes Oligodendrocytes (CNS myelin)	**Muscle** Smooth Cardiac Skeletal Connective tissue All serous membranes Bone and cartilage Blood, lymph, cardiovascular organs Adrenal cortex Gonads and internal reproductive organs Spleen Kidney and ureter Dura mater	**Forms epithelial parts of:** Tonsils Thymus Pharynx Larynx Trachea Bronchi Lungs Urinary bladder Urethra Tympanic cavity Auditory tube GI tract
Ectoderm	**Mesoderm**	**Endoderm**
Neural crest Adrenal medulla Ganglia Sensory (unipolar) Autonomic (postganglionic) Pigment cells (melanocytes) Schwann cells (PNS myelin) Meninges Pia and arachnoid mater Pharyngeal arch cartilage (first arch syndromes) Odontoblasts Parafollicular (C) cells Aorticopulmonary septum (tetralogy of Fallot) Endocardial cushions (Down syndrome)		**Forms parenchyma of:** Liver Pancreas Tonsils Thyroid gland Parathyroid glands Glands of the GI tract Submandibular gland Sublingual gland

Yolk sac derivatives:

Primordial germ cells

Early blood and blood vessels

AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) is responsible for the motor innervation of smooth muscle, cardiac muscle, and glands of the body. It is divided into the **sympathetic** and **parasympathetic** nervous systems. In both systems there are 2 neurons in the peripheral distribution of the motor innervation.

- Preganglionic neuron with cell body in CNS
- Postganglionic neuron with cell body in a ganglion in the PNS

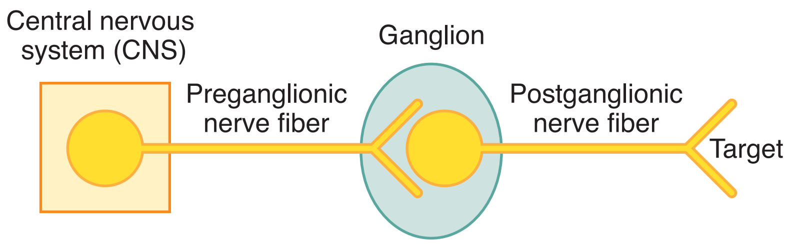


Figure III-1-3. Autonomic Nervous System

Peripheral Nervous System

N_N: neuronal nicotinic receptor

N_M: muscle nicotinic receptor

NE: norepinephrine

M: muscarinic receptor

ACh: acetylcholine

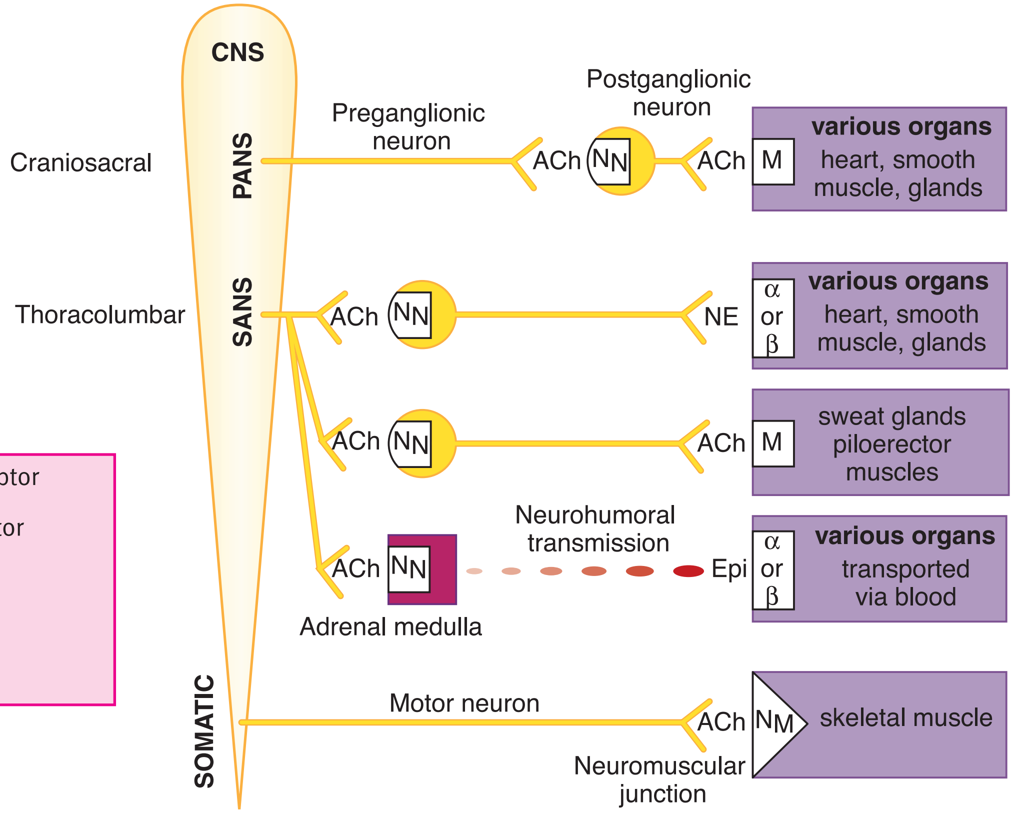


Figure III-1-4. Autonomic and Somatic Nervous System Neurotransmitters/Receptors

- **Somatic nervous system:** 1 neuron (from CNS → effector organ)
- **ANS:** 2 neurons (from CNS → effector organ)
  - **Preganglionic** neuron: cell body in CNS
  - **Postganglionic** neuron: cell body in ganglia in PNS
  - **Parasympathetic:** long preganglionic, short postganglionic
  - **Sympathetic:** short preganglionic, long postganglionic (except adrenal medulla)

Parasympathetic Nervous System

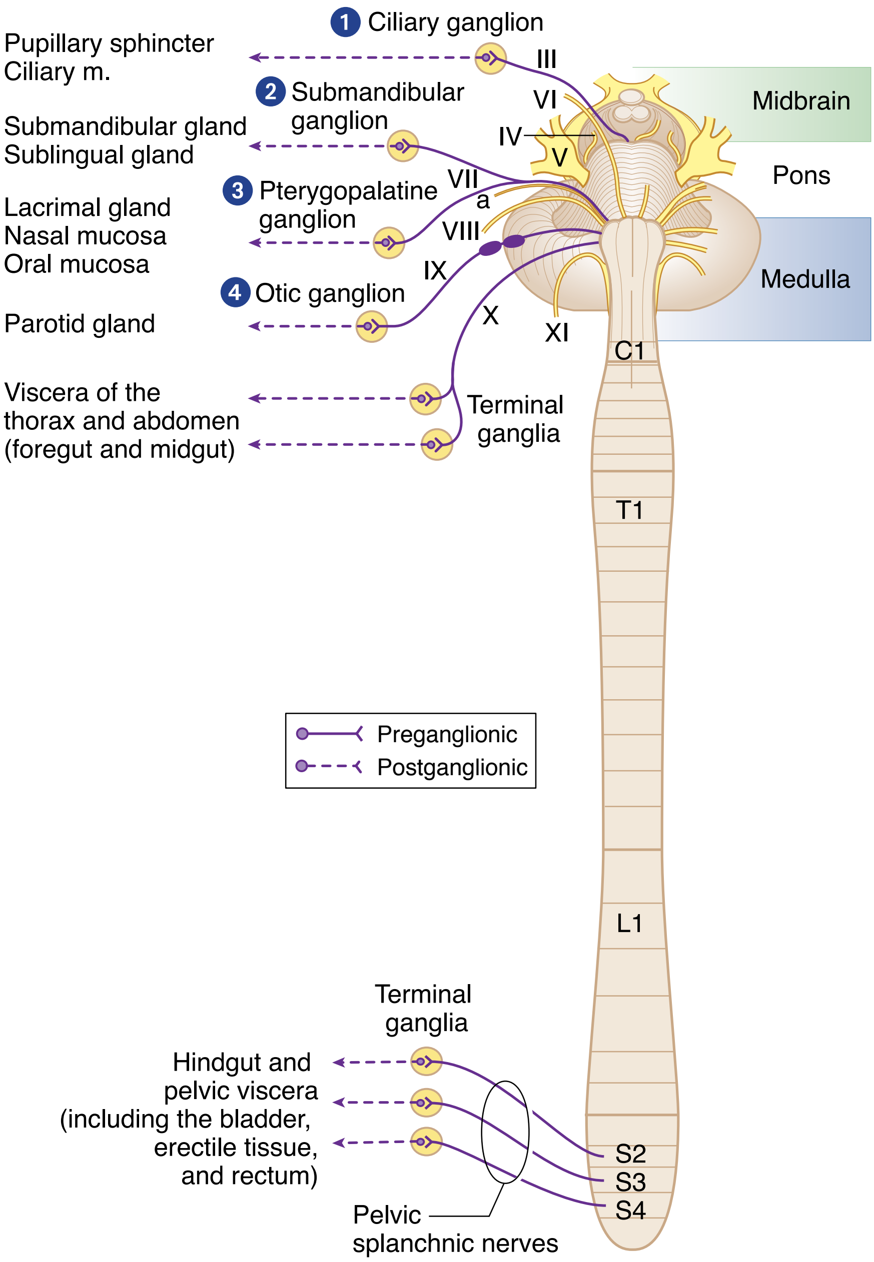


Figure III-1-5. Overview of Parasympathetic Outflow

Clinical Correlate

Hirschsprung's disease results from missing terminal ganglia in the wall of the rectum. Infants cannot pass meconium.

Table III-1-4. Parasympathetic = Craniosacral Outflow

Origin	Site of Synapse	Innervation
Cranial nerves III, VII, IX	Four cranial ganglia	Glands and smooth muscle of the head
Cranial nerve X	Terminal ganglia (in or near the walls of viscera)	Viscera of the neck, thorax, foregut, and midgut
Pelvic splanchnic nerves (S2, S3, S4)	Terminal ganglia (in or near the walls of viscera)	Hindgut and pelvic viscera (including bladder and erectile tissue)

Note

Horner's syndrome results in *ipsilateral* ptosis, miosis, anhydrosis.

Sympathetic Nervous System

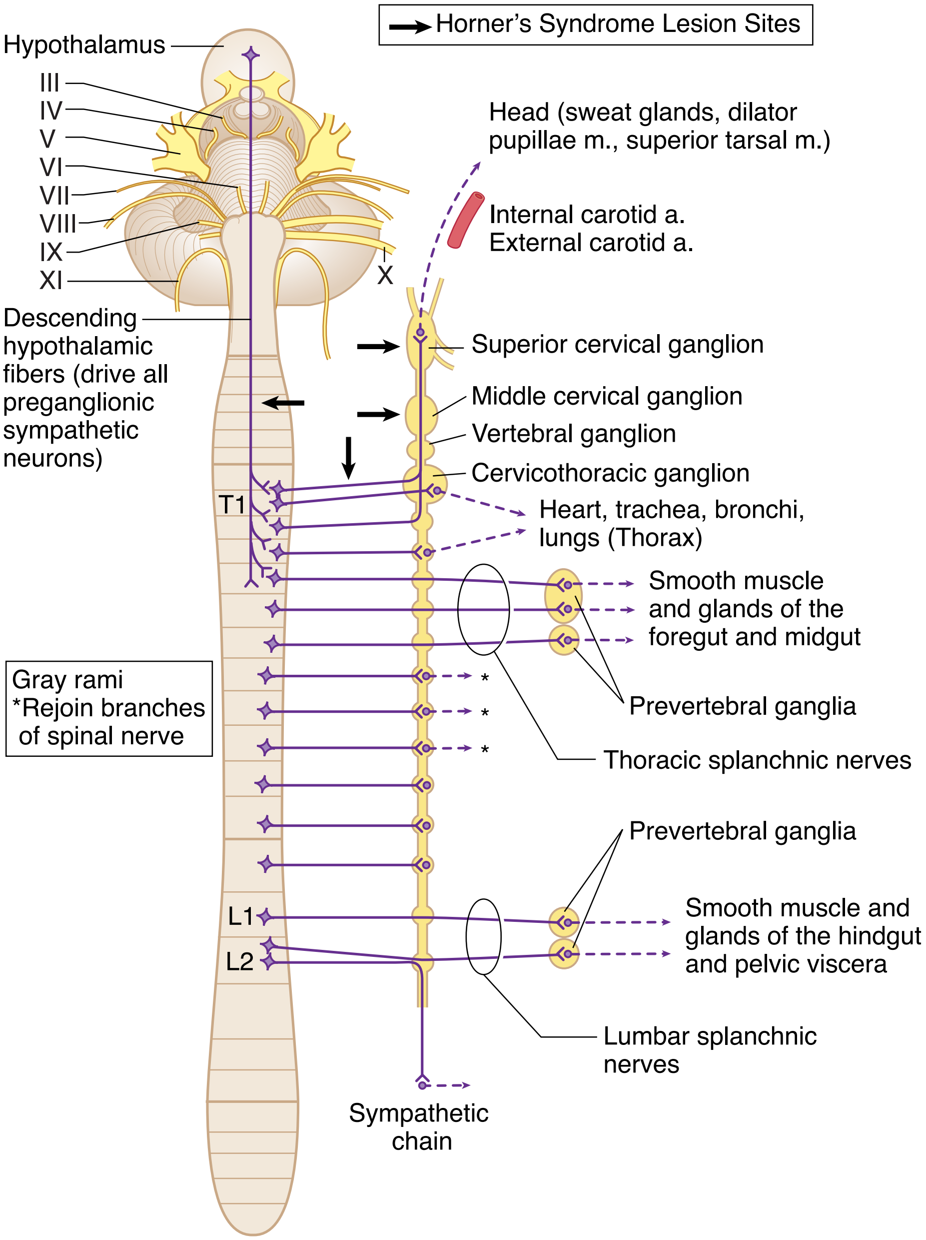


Figure III-1-6. Overview of Sympathetic Outflow

Table III-1-5. Sympathetic = Thoracolumbar Outflow

Origin	Site of Synapse	Innervation
Spinal cord levels T1–L2	Sympathetic chain ganglia (paravertebral ganglia)	Smooth muscle and glands of body wall and limbs; head and thoracic viscera
Thoracic splanchnic nerves T5–T12	Prevertebral ganglia (collateral; e.g., celiac, aorticorenal superior mesenteric ganglia)	Smooth muscle and glands of the foregut and midgut
Lumbar splanchnic nerves L1, L2	Prevertebral ganglia (collateral; e.g., inferior mesenteric and pelvic ganglia)	Smooth muscle and glands of the pelvic viscera and hindgut

Note

Gray rami are postganglionics that rejoin spinal nerves to go to the body wall.

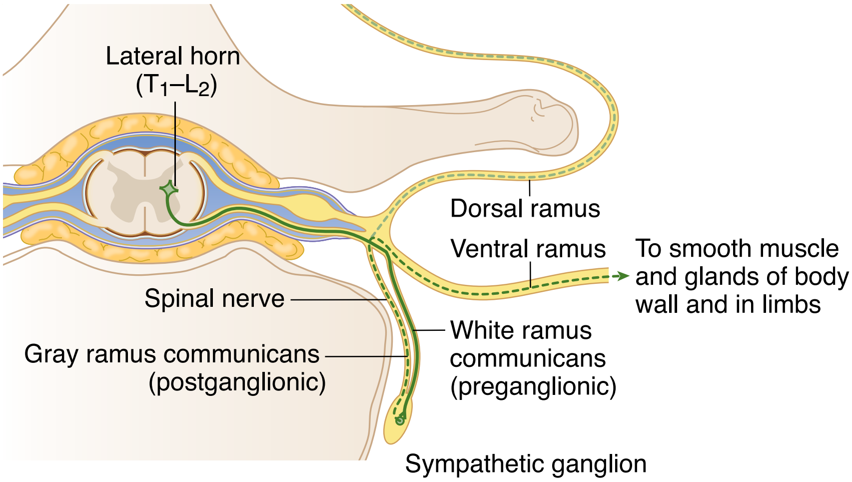


Figure III-1-7. Cross Section of Spinal Cord Showing Sympathetic Outflow

Histology of the Nervous System

2

Learning Objectives

- ❏ Explain information related to neurons
- ❏ Solve problems concerning disorders of myelination

NEURONS

Neurons are cells which are morphologically and functionally polarized so that information may pass from one end of the cell to the other. Neurons may be classified by the form and number of their processes as bipolar, unipolar, or multipolar. The cell body of the neuron contains the nucleus and membrane-bound cytoplasmic organelles typical of a eukaryotic cell, including endoplasmic reticulum (ER), Golgi apparatus, mitochondria, and lysosomes. The nucleus and nucleolus are prominent in neurons.

The cytoplasm contains Nissl substance, clumps of rough ER with bound polysomes. The cytoplasm also contains free polysomes; free and bound polysomes in the Nissl substance are sites of protein synthesis.

Multipolar Neuron

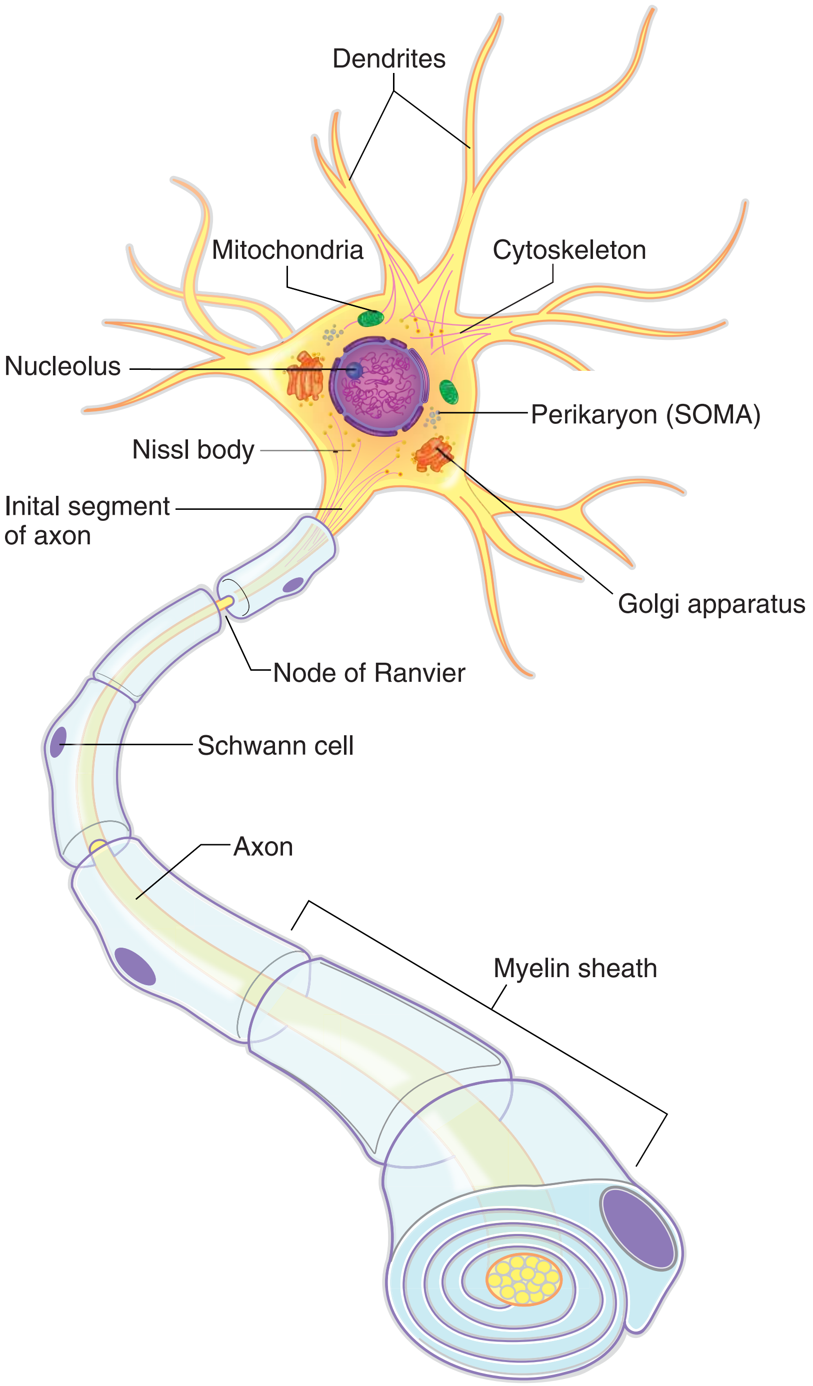


Figure III-2-1. Neuron Structure

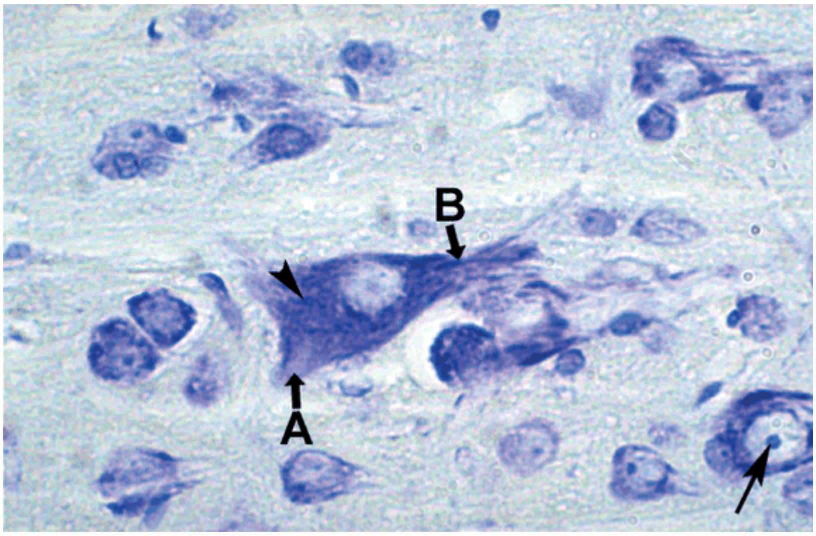


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Figure III-2-2. Neural Tissue with Nissl stain that stains rough ER in cell body (arrowhead) and proximal parts of dendrites (B)

The axon (A) lacks Nissl substance. The nucleus of an adjacent neuron has a prominent nucleolus (arrow).

Cytoskeleton

The **cytoskeleton of the neuron** consists of neurofilaments, microfilaments, and microtubules.

- **Neurofilaments** provide structural support for the neuron, and are most numerous in the axon and the proximal parts of dendrites.
- **Microfilaments** form a matrix near the periphery of the neuron. A microfilament matrix is prominent in growth cones of neuronal processes and functions to aid in the motility of growth cones during development. A microfilament matrix is also prominent in dendrites and forms structural specializations at synaptic membranes.
- **Microtubules** are found in all parts of the neuron, and are the cytoplasmic organelles used in axonal transport.

Clinical Correlate

CNS Disease and Cytoplasmic Inclusions in Neurons

Lewy bodies are cytoplasmic inclusions of degenerating neurons of the substantia nigra, pars compacta, evident in Parkinson's disease and in cortical and brain-stem neurons seen in certain forms of dementia.

Negri bodies are eosinophilic cytoplasmic inclusions seen in degenerating neurons in the hippocampus and cerebellar cortex in patients with rabies.

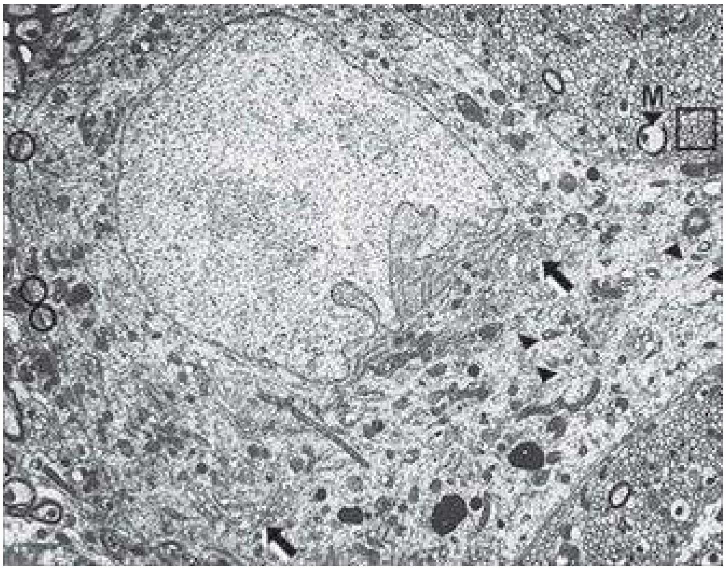

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Figure III-2-3. EM of Neuropil including Cell Body of a Neuron with Rough ER (arrowheads) and Golgi (arrows)

Surrounding neuropil has myelinated axons(M) and unmyelinated bare axons (box).

Clinical Correlate

In degenerative neuronal diseases of the CNS, a **tau protein** becomes excessively phosphorylated, which prevents crosslinking of microtubules. The affected microtubules form helical filaments and neurofibrillary tangles and senile plaques in the cell body and dendrites of neurons. **Neurofibrillary tangles** are prominent features of degenerating neurons in Alzheimer's disease, amyotrophic lateral sclerosis, and Down syndrome.

Dendrites taper from the cell body and provide the major surface for synaptic contacts with axons of other neurons. **Dendrites may contain spines**, which are small cytoplasmic extensions that dramatically increase the surface area of dendrites. Dendrites may be highly branched; the branching pattern of dendrites may be used to define a particular neuronal cell type.

The **axon** has a **uniform diameter and may branch at right angles into collaterals** along the length of the axon, in particular near its distal end. The proximal part of the axon is usually marked by an **axon hillock**, a tapered extension of the cell body that lacks Nissl substance.

The initial segment is adjacent to the axon hillock. The membrane of the initial segment contains numerous voltage-sensitive sodium ion channels. The initial segment is the "trigger zone" of an axon where conduction of electrical activity as an action potential is initiated.

If the axon is myelinated, the myelin sheath begins at the initial segment. The cytoplasm of the entire axon lacks free polysomes, Nissl substance, and Golgi apparatus but contains mitochondria and smooth ER.

Anterograde axonal transport moves proteins and membranes that are synthesized in the cell body through the axon to the synaptic terminal. In **fast anterograde transport**, there is a rapid (100–400 mm/day) movement of materials from the cell body to the axon terminal.

- Fast anterograde transport is dependent on **kinesin,** which acts as the motor molecule. Fast anterograde transport delivers precursors of peptide neurotransmitters to synaptic terminals.

- In **slow anterograde transport**, there is a slow (1–2 mm/day) anterograde movement of soluble cytoplasmic components. Cytoskeletal proteins, enzymes, and precursors of small molecule neurotransmitters are transported to synaptic terminals by slow anterograde transport. Slow transport is not dependent on microtubules or ATPase motor molecules.

Clinical Correlate

Neuropathies and Axonal Transport

- Disruption of fast anterograde transport may result in an axonal polyneuropathy. The cause may be anoxia (which affects mitochondrial oxidative phosphorylation) or anticancer agents e.g., colchicine and vinblastine (which depolymerize microtubules).
- In patients with diabetes, hyperglycemia results in an alteration of proteins that form microtubules, which may disrupt axonal transport. Patients may develop axonal polyneuropathies in long axons in nerves, producing a "glove-and-stocking" pattern of altered sensation and pain in the feet and then in the hands.

Retrograde axonal transport returns intracellular material from the synaptic terminal to the cell body to be recycled or digested by lysosomes.

Retrograde transport uses microtubules and is slower than anterograde transport (60–100 mm/day). It is dependent on dynein, an ATPase, which acts as the retrograde motor molecule. Retrograde transport also permits communication between the synaptic terminal and the cell body by transporting trophic factors emanating from the postsynaptic target or in the extracellular space.

Glial and Supporting Cells in the CNS and PNS

The supporting (or glial) cells of the CNS are small cells that differ from neurons. Supporting cells have only one kind of process and do not form chemical synapses. Unlike neurons, they readily divide and proliferate; glioma is the most common type of primary tumor of the CNS.

Astrocytes are the **most numerous glial cells** in the CNS, and they have large numbers of radiating processes.

- They provide the structural support or scaffolding for the CNS and contain large bundles of intermediate filaments that consist of **glial fibrillary acidic protein** (GFAP).
- They have uptake systems which remove the neurotransmitter glutamate and K^+ ions from the extracellular space.
- They have foot processes which contribute to the blood–brain barrier by forming a glial-limiting membrane.
- They hypertrophy and proliferate after an injury to the CNS, filling up the extracellular space left by degenerating neurons by forming an astroglial scar.

Clinical Correlate

Retrograde Axonal Transport and Neurological Disorders

The polio, herpes, and rabies viruses and tetanus toxins are taken up and retrogradely transported by axons that innervate skeletal muscle.

Herpes is taken up and retrogradely transported in sensory fibers and remains dormant in sensory ganglia.

Radial glia are precursors of astrocytes that guide neuroblast migration during CNS development.

Microglia cells are the **smallest glial cells** in the CNS. Unlike the rest of the CNS neurons and glia, which are derived from neuroectoderm, microglia are derived from bone marrow monocytes and enter the CNS after birth.

- They provide a link between cells of the CNS and the immune system.
- They proliferate and migrate to the site of a CNS injury and phagocytose neuronal debris after injury. Pericytes are microglia which contribute to the blood–brain barrier.
- They determine the chances of survival of a CNS tissue graft, and are the cells in the CNS that are targeted by the HIV-1 virus in those with AIDS. The affected microglia may produce cytokines that are toxic to neurons.

CNS microglia that become phagocytic in response to neuronal tissue damage may secrete toxic free radicals. Accumulation of free radicals, such as superoxide, may lead to disruption of the calcium homeostasis of neurons.

Oligodendrocytes form myelin for axons in the CNS. Each of the processes of the oligodendrocyte can myelinate individual segments of many axons. Unmyelinated axons in the CNS are not ensheathed by oligodendrocyte cytoplasm.

Schwann cells are the supporting cells of the peripheral nervous system (PNS), and are derived from neural crest cells. Schwann cells form the myelin for axons and processes in the PNS. Each Schwann cell forms myelin for only a single internodal segment of a single axon. Unmyelinated axons in the PNS are enveloped by the cytoplasmic processes of a Schwann cell. Schwann cells act as phagocytes and remove neuronal debris in the PNS after injury. A **node of Ranvier** is the region between adjacent myelinated segments of axons in the CNS and the PNS.

In all myelinated axons, nodes of Ranvier are sites that permit action potentials to jump from node to node (saltatory conduction). Saltatory conduction dramatically increases the conduction velocity of impulses in myelinated axons.

DISORDERS OF MYELINATION

Demyelinating diseases are acquired conditions involving selective damage to myelin. Other diseases (e.g., infectious, metabolic, inherited) can also affect myelin and are generally called leukodystrophies.

Table III-2-1. Conditions of Impaired Myelination

Disease	Symptoms	Notes
Multiple sclerosis (MS)	Symptoms separated in space and time Vision loss (optic neuritis) Internuclear ophthalmoplegia (MLF) Motor and sensory deficits Vertigo Neuropsychiatric	Occurs 2x more often in women Onset often in decades 3 or 4 Higher prevalence in temperate zones Relapsing–remitting course is most common Well-circumscribed demyelinated plaques often in periventricular areas Chronic inflammation; axons initially preserved Increased IgG (oligoclonal bands) in CSF Treatment: high-dose steroids, interferon-beta, glatiramer (Copaxone®)
Metachromatic leukodystrophy (MLD)	Four types; motor and cognitive issues with seizures	Arylsulfatase A deficiency in lysosomes affects both CNS and PNS myelin
Progressive multifocal leukoencephalopathy (PML)	Cortical myelin commonly affected; causes limb weakness, speech problems	Caused by JC virus Affects immunocompromised, especially AIDS Demyelination, astrogliosis, lymphohistiocytosis
Central pontine myelinolysis (CPM)	Pseudobulbar palsy Spastic quadriparesis Mental changes May produce the "locked-in" syndrome Often fatal	Focal demyelination of central area of basis pontis (affects corticospinal, corticobulbar tracts) Seen in severely malnourished, alcoholics, liver disease Probably caused by overly aggressive correction of hyponatremia
Guillain-Barré syndrome	Acute symmetric ascending inflammatory neuropathy of PNS myelin Weakness begins in lower limbs and ascends; respiratory failure can occur in severe cases Autonomic dysfunction may be prominent Cranial nerve involvement is common Sensory loss, pain, and paresthesias rarely occur Reflexes invariably decreased or absent	Two-thirds of patients have history of respiratory or GI illness 1–3 weeks prior to onset Elevated CSF protein with normal cell count (albuminocytologic dissociation)

Abbreviations: JC, John Cunningham; MLF, medial longitudinal fasciculus

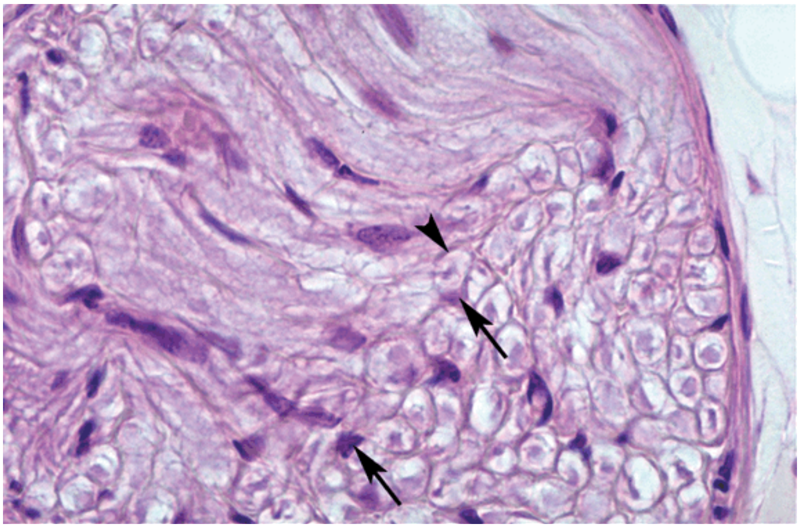

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Figure III-2-4. Section of a Peripheral Nerve

Note endoneurial border of an individual axon (arrowhead) and 2 Schwann cell nuclei (arrows)

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Figure III-2-5. Axons Cut in Cross-Section

Ependymal cells line the ventricles in the adult brain. Some ependymal cells differentiate into choroid epithelial cells, forming part of the choroid plexus, which produces cerebrospinal fluid (CSF). Ependymal cells are ciliated; ciliary action helps circulate CSF.

Tanycytes are specialized ependymal cells that have basal cytoplasmic processes in contact with blood vessels; these processes may transport substances between a blood vessel and a ventricle.

Blood–Brain Barrier

The blood–brain barrier restricts access of micro-organisms, proteins, cells, and drugs to the nervous system. It consists of capillary endothelial cells, an underlying basal lamina, astrocytes, and pericytes.

- The most important elements of the blood-brain barrier are the **cerebral capillary endothelial cells** and their **intercellular tight junctions**.
- Astrocytes and pericytes are found at the blood–brain barrier outside the basal lamina. Astrocytes have processes with "end feet" that cover >95% of the basal lamina adjacent to the capillary endothelial cells.

Substances cross the blood–brain barrier into the CNS by **diffusion, by selective transport, and via ion channels.** Oxygen and carbon dioxide are lipid-soluble gases that readily diffuse across the blood–brain barrier. Glucose, amino acids, and vitamins K and D are selectively transported across the blood–brain barrier. Sodium and potassium ions move across the blood–brain barrier through ion channels.

Clinical Correlate

Drugs of addiction—heroin, ethanol, and nicotine—are lipid-soluble compounds that readily diffuse across the blood–brain barrier.

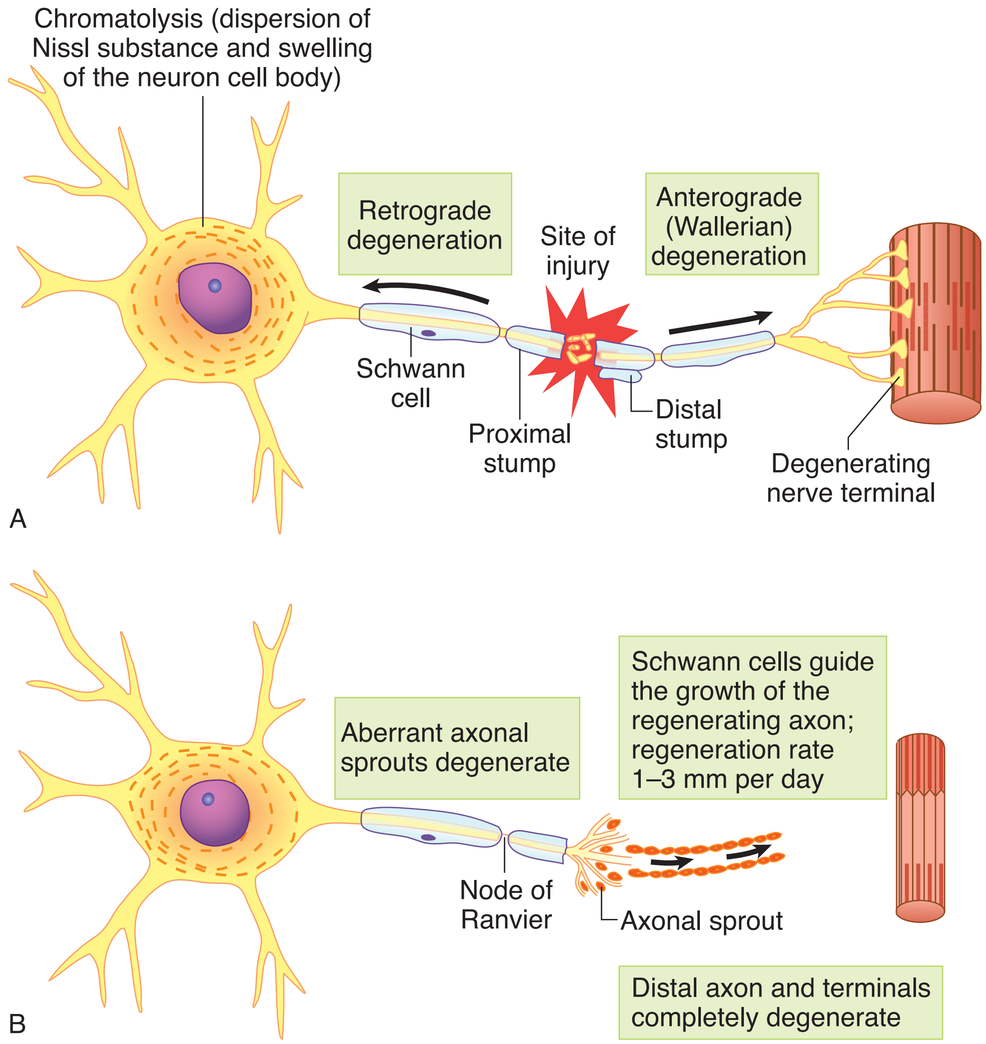


Figure III-2-6. Neuron Undergoing Axotomy, Chromatolysis, and Regeneration in the PNS

Response of Axons to Destructive Lesions (Severing an Axon or Axotomy) or Irritative Lesions (Compression of an Axon)

Anterograde or Wallerian degeneration distal to the cut occurs when an axon is severed in either the CNS or PNS. The closer the destructive lesion is to the neuronal cell body, the more likely the neuron is to die. In the PNS, anterograde degeneration of axons is rapid and complete after several weeks. In the PNS, the endoneurial Schwann cell sheath that envelops a degenerating axon does not degenerate and provides a scaffold for regeneration and remyelination of the axon.

Clinical Correlate

Schwannomas typically affect VIII nerve fibers seen in neurofibromatosis type 2.

Neurons with severed axons in the PNS are capable of complete axonal regeneration. Successful sprouts from the cut axon grow into and through endoneurial sheaths and are guided by Schwann cells back to their targets. Regeneration proceeds at the rate of 1–2 mm/day, which corresponds to the rate of slow anterograde transport.

Half of brain and spinal cord tumors are metastatic.

Table III-2-2. Primary Tumors

Tumor	Features	Pathology
Glioblastoma multiforme (grade IV astrocytoma)	**Most common primary brain tumor** • **Highly malignant** • Usually lethal in 8–12 months	• Can cross the midline via the corpus callosum (**"butterfly glioma"**) • Areas of necrosis surrounded by rows of neoplastic cells (**pseudopalisading necrosis**)
Astrocytoma (pilocytic)	• Benign tumor of children and young adults • Usually in **posterior fossa in children**	• **Rosenthal fibers** • Immunostaining with **GFAP**
Oligodendroglioma	• Slow growing • Long survival (average 5–10 years)	• **"Fried-egg" appearance**—perinuclear halo
Ependymoma	• Ependymal origin • Can arise in IV ventricle and lead to **hydrocephalus**	• Rosettes and pseudorosettes
Medulloblastoma	• Highly malignant cerebellar tumor • A type of primitive neuroectodermal tumor (PNET)	• Blue, small, round cells with pseudorosettes
Meningioma	• Second most common primary brain tumor • Dural convexities; parasagittal region	• Attaches to the dura, compresses underlying brain without invasion • Microscopic—**psammoma bodies**
Schwannoma	• Third most common primary brain tumor • Most frequent location: **CN VIII at cerebellopontine angle** • **Hearing loss, tinnitus, CN V + VII signs** • Good prognosis after surgical resection	• Antoni A (hypercellular) and B (hypocellular) areas • **Bilateral acoustic schwannomas—pathognomonic for neurofibromatosis type 2**
Retinoblastoma	• Sporadic—unilateral • **Familial—bilateral; associated with osteosarcoma**	• Small, round, blue cells; may have rosettes
Craniopharyngioma	• Derived from oral epithelium (remnants of **Rathke pouch**) • Usually children and young adults • Often calcified • Symptoms due to encroachment on pituitary stalk or **optic chiasm** • Benign but may recur	• Histology resembles **adamantinoma** (most common tumor of tooth)

Abbreviation: GFAP, glial fibrillary acidic protein

Ventricular System 3

Learning Objectives

- ❑ Demonstrate understanding of ventricular system and venous drainage
- ❑ Explain information related to CSF distribution, secretion, and circulation

The brain and spinal cord float within a protective bath of cerebrospinal fluid (CSF), which is produced continuously by the choroid plexus within the ventricles of the brain.

Each part of the CNS contains a component of the ventricular system. There are 4 interconnected ventricles in the brain: 2 lateral ventricles, a third ventricle, and a fourth ventricle.

- A lateral ventricle is located deep within each cerebral hemisphere. Each lateral ventricle communicates with the third ventricle via an interventricular foramen (foramen of Monro).
- The third ventricle is found in the midline within the diencephalon and communicates with the fourth ventricle via the cerebral aqueduct (of Sylvius), which passes through the midbrain.
- The fourth ventricle is located between the dorsal surfaces of the pons and upper medulla and the ventral surface of the cerebellum. The fourth ventricle is continuous with the central canal of the lower medulla and spinal cord.

Note

Choroid plexus secretes CSF into all ventricles. Arachnoid granulations are sites of CSF resorption.

VENTRICULAR SYSTEM AND VENOUS DRAINAGE

The brain and spinal cord float within a protective bath of CSF, which is produced by the lining of the ventricles, the choroid plexus. CSF circulation begins in the ventricles and then enters the subarachnoid space to surround the brain and spinal cord.

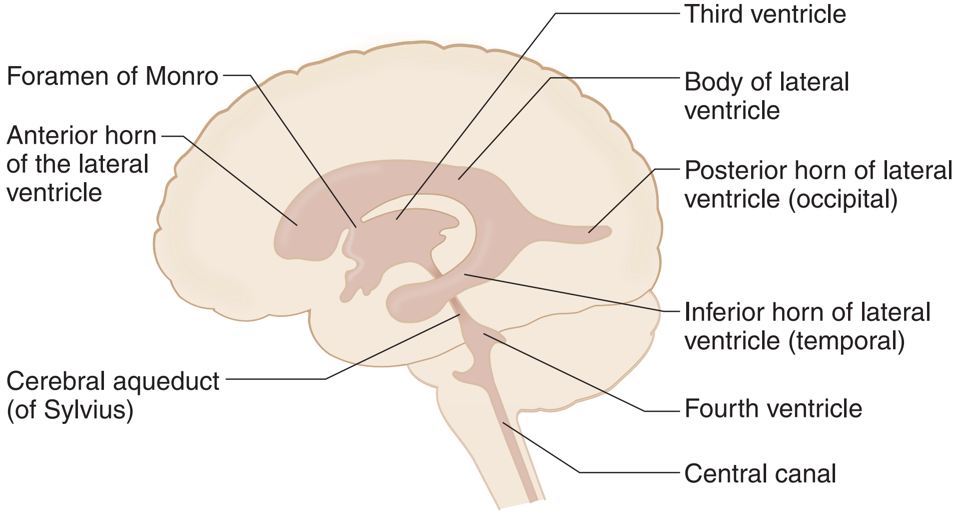


Figure III-3-1. Ventricles and CSF Circulation

Note

A total of 400–500 cc of CSF is produced per day; ventricles and subarachnoid space contain 90–150 cc, so all of CSF is turned over 2–3 times per day.

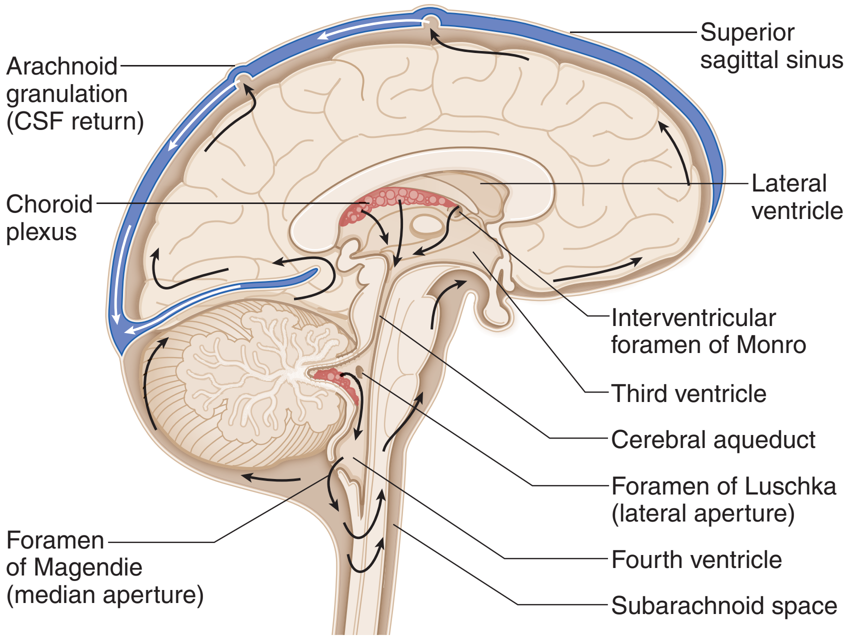


Figure III-3-2. Sagittal Section of the Brain

Lateral ventricles $\xrightarrow{\text{interventricular foramen of Monro}}$ third ventricle $\xrightarrow{\text{cerebral aqueduct}}$ fourth ventricle → subarachnoid space (via foramina of Luschka and foramen of Magendie)

CSF Production and Barriers

Choroid plexus—contains **choroid epithelial cells** and is in the lateral, third, and fourth ventricles. **Secretes CSF.** Tight junctions form **blood-CSF barrier**.

Blood-brain barrier—formed by capillary endothelium with tight junctions; astrocyte foot processes contribute.

Once CSF is in the subarachnoid space, it goes up over convexity of the brain and enters the venous circulation by passing through **arachnoid granulations** into **dural venous sinuses**.

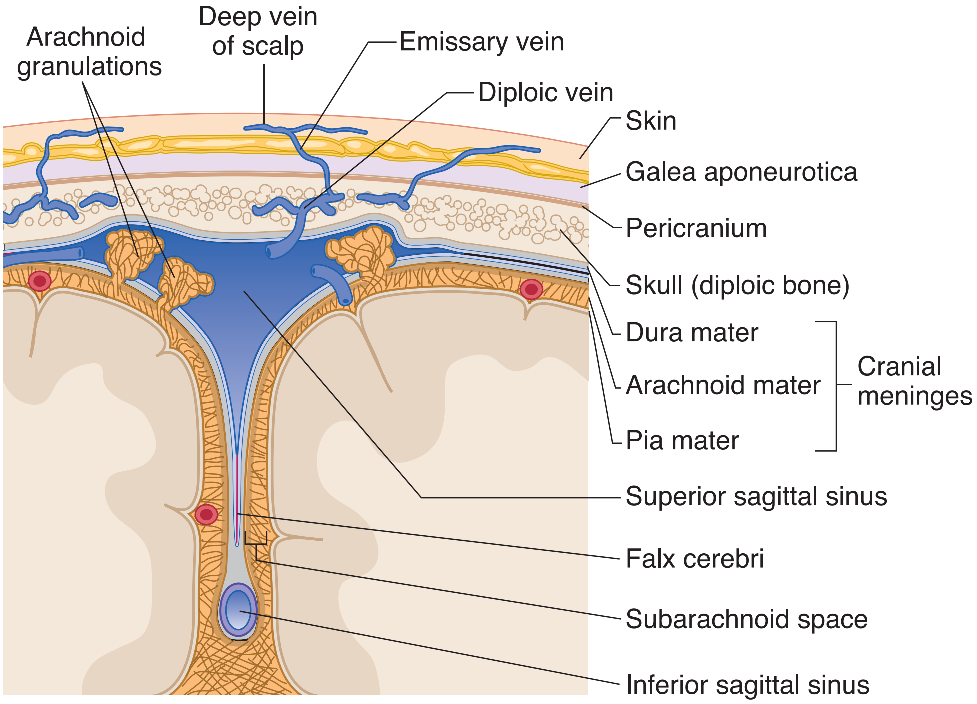


Figure III-3-3. Coronal Section of the Dural Sinuses

Sinuses

Superior sagittal sinus (in superior margin of falx cerebri)—drains into 2 **transverse sinuses**. Each of these drains blood from the **confluence of sinuses** into **sigmoid sinuses**. Each sigmoid sinus exits the skull (via **jugular foramen**) as the **internal jugular veins**.

Inferior sagittal sinus (in inferior margin of falx cerebri)— terminates by joining with the great cerebral vein of Galen to form the **straight sinus** at the falx cerebri and tentorium cerebelli junction. This drains into the confluence of sinuses.

Cavernous sinus—a plexus of veins on either side of the **sella turcica**. Surrounds internal carotid artery and cranial nerves III, IV, V, and VI. It drains into the transverse sinus (via the **superior petrosal sinus**) and the internal jugular vein (via the **inferior petrosal sinus**).

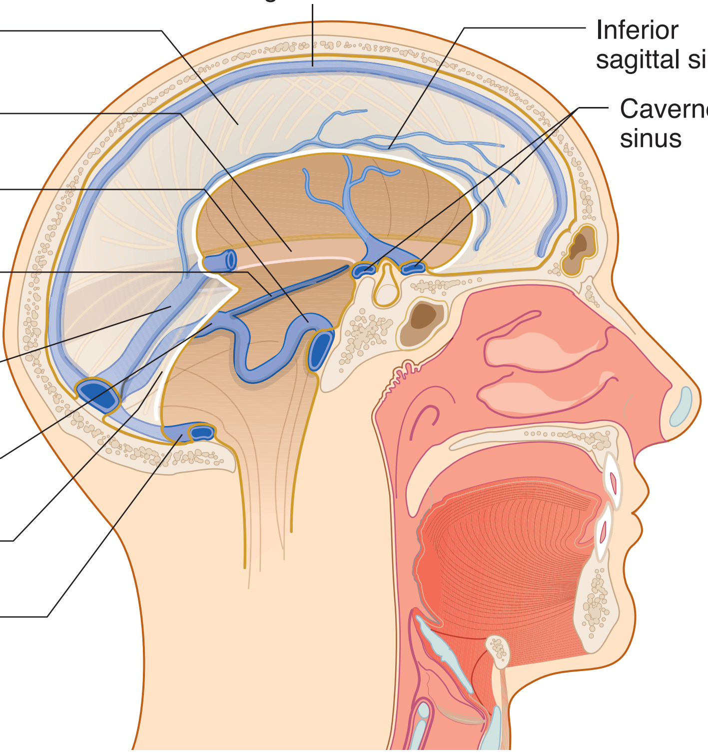


Figure III-3-4. Dural Venous Sinuses

Hydrocephalus

Excess volume or pressure of CSF, leading to dilated ventricles

Table III-3-1. Types and Features of Hydrocephalus

Type of Hydrocephalus	Description
Noncommunicating	**Obstruction of flow within ventricles;** most commonly occurs at narrow points, e.g., foramen of Monro, cerebral aqueduct and/or openings of fourth ventricle
Communicating	**Impaired CSF reabsorption** in arachnoid granulations or obstruction of flow in subarachnoid space
Normal pressure (chronic)	CSF is not absorbed by arachnoid villi (a form of communicating hydrocephalus). CSF pressure is usually normal. Ventricles chronically dilated. Produces **triad** of **dementia, apraxic (magnetic) gait,** and **urinary incontinence.** Peritoneal shunt.
Ex vacuo	Descriptive term referring to excess CSF in regions where brain tissue is lost due to atrophy, stroke, surgery, trauma, etc. Results in dilated ventricles but normal CSF pressure.

CSF DISTRIBUTION, SECRETION, AND CIRCULATION

CSF fills the subarachnoid space and the ventricles of the brain. The average adult has 90–150 mL of total CSF, although 400–500 mL is produced daily. Only 25 mL of CSF is found in the ventricles themselves.

Approximately 70% of the CSF is secreted by the choroid plexus, which consists of glomerular tufts of capillaries covered by ependymal cells that project into the ventricles (the remaining 30% represents metabolic water production). The choroid plexus is located in parts of each lateral ventricle, the third ventricle, and the fourth ventricle.

CSF from the lateral ventricles passes through the interventricular foramina of Monro into the third ventricle. From there, CSF flows through the aqueduct of Sylvius into the fourth ventricle. The only sites where CSF can leave the ventricles and enter the subarachnoid space outside the CNS are through 3 openings in the fourth ventricle, 2 lateral foramina of Luschka and the median foramen of Magendie.

Within the subarachnoid space, CSF also flows up over the convexity of the brain and around the spinal cord. Almost all CSF returns to the venous system by draining through arachnoid granulations into the superior sagittal dural venous sinus.

- Normal CSF is a **clear** fluid, isotonic with serum (290–295 mOsm/L).
- The pH of CSF is 7.33 (arterial blood pH, 7.40; venous blood pH, 7.36).
- Sodium ion (Na^+) concentration is equal in serum and CSF (≈138 mEq/L).
- CSF has a higher concentration of chloride (Cl^-) and magnesium (Mg^{2+}) ions than does serum.
- CSF has a lower concentration of potassium (K^+), calcium (Ca^{2+}), and bicarbonate (HCO_3^-) ions, as well as glucose, than does serum.

The **blood–CSF barrier** is a mechanism which protects the chemical integrity of the brain. Tight junctions located along the epithelial cells of the choroid plexus form the blood–CSF barrier. Transport systems are similar but not identical to the those of blood–brain barrier. The ability of a substance (drug) to enter the CSF does not guarantee it will gain access to the brain.

Clinical Correlate

The concentration of protein (including all immunoglobulins) is much lower in the CSF as compared with serum.

Normal CSF contains 0–4 lymphocytes or mononuclear cells per cubic millimeter. Although the presence of a few monocytes or lymphocytes is normal, the presence of polymorphonuclear leukocytes is always abnormal, as in bacterial meningitis.

- Red blood cells are not normally found in the CSF but may be present after traumatic spinal tap or subarachnoid hemorrhage.
- Increased protein levels may indicate a CNS tumor.
- Tumor cells may be present in the CSF in cases with meningeal involvement.

The Spinal Cord 4

Learning Objectives

- ❏ Solve problems concerning general features
- ❏ Interpret scenarios on neural systems

GENERAL FEATURES

The spinal cord is housed in the vertebral canal. It is continuous with the medulla below the pyramidal decussation and terminates as the conus medullaris at the second lumbar vertebra of the adult. The roots of 31 pairs of spinal nerves arise segmentally from the spinal cord.

There are **8 cervical pairs** of spinal nerves (C1 through C8). The cervical enlargement (C5 through T1) gives rise to the rootlets that form the brachial plexus, which innervates the upper limbs.

There are **12 thoracic pairs** of spinal nerves (T1 through T12). Spinal nerves emanating from thoracic levels innervate most of the trunk.

There are **5 lumbar pairs** of spinal nerves (L1 through L5). The lumbar enlargement (L2 through S3) gives rise to rootlets that form the lumbar and sacral plexuses, which innervate the lower limbs.

There are **5 sacral pairs** of spinal nerves (S1 through S5). Spinal nerves at the sacral level innervate part of the lower limbs and the pelvis.

There is **1 coccygeal pair** of spinal nerves. The cauda equina consists of the dorsal and ventral roots of the lumbar, sacral, and coccygeal spinal nerves.

Inside the spinal cord, gray matter is centrally located and shaped like a butterfly. It contains neuronal cell bodies, their dendrites, and the proximal parts of axons. White matter surrounds the gray matter on all sides. White matter contains bundles of functionally similar axons called tracts or fasciculi, which ascend or descend in the spinal cord.

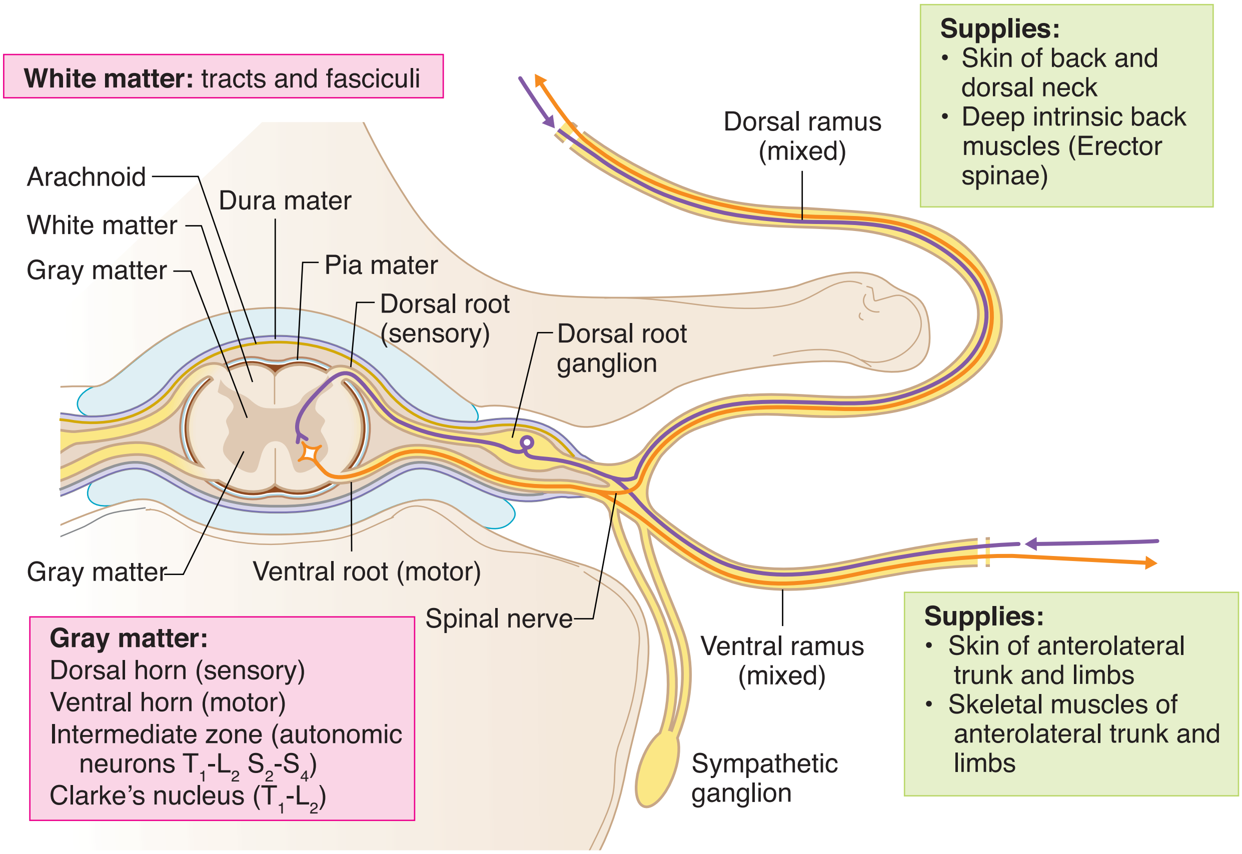


Figure III-4-1. Cross Section of Spinal Cord and Parts of Spinal Nerve

Table III-4-1. General Spinal Cord Features

Conus medullaris	Caudal end of the spinal cord (S3–S5). In adult, ends at the L2 vertebra
Cauda equina	Nerve roots of the lumbar, sacral, and coccygeal spinal nerves
Filum terminale	Slender pial extension that tethers the spinal cord to the bottom of the vertebral column
Doral root ganglia	Cell bodies of primary sensory neurons
Dorsal and ventral roots	Each segment has a pair
Dorsal horn	**Sensory neurons**
Ventral horn	**Motor neurons**
Spinal nerve	Formed from dorsal and ventral roots (mixed nerve)
Cervical enlargement	(C5–T1) → branchial plexus → upper limbs
Lumbar enlargement	(L2–S3) → lumbar and sacral plexuses → lower limbs

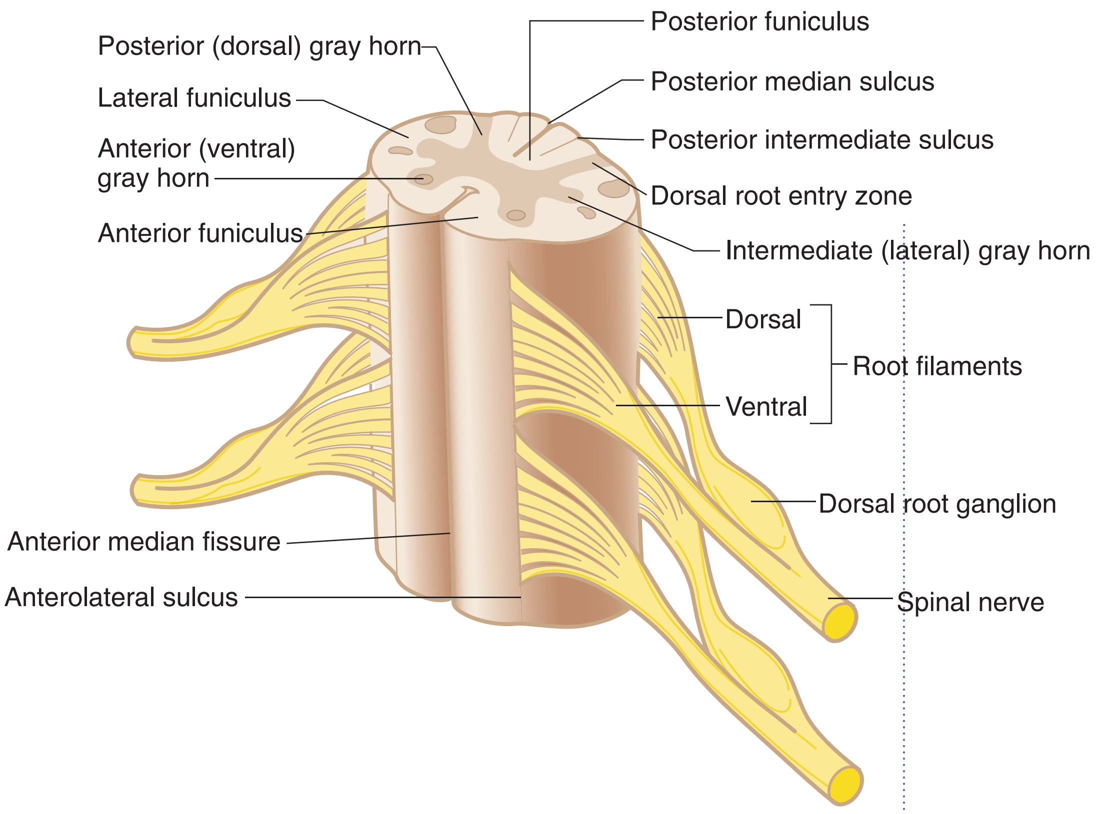


Figure III-4-2. General Spinal Cord Features

Dorsal Horn

The dorsal horn is dominated by neurons that respond to sensory stimulation. All incoming sensory fibers in spinal nerves enter the dorsolateral part of the cord adjacent to the dorsal horn in a dorsal root. Neurons in the dorsal horn project to higher levels of the CNS to carry sensations to the brain stem, cerebral cortex, or cerebellum. Other dorsal horn neurons participate in reflexes.

Dorsal horn: rexed laminae I–VI

Ventral horn: rexed laminae VIII–IX

Intermediate zone: lamina VII

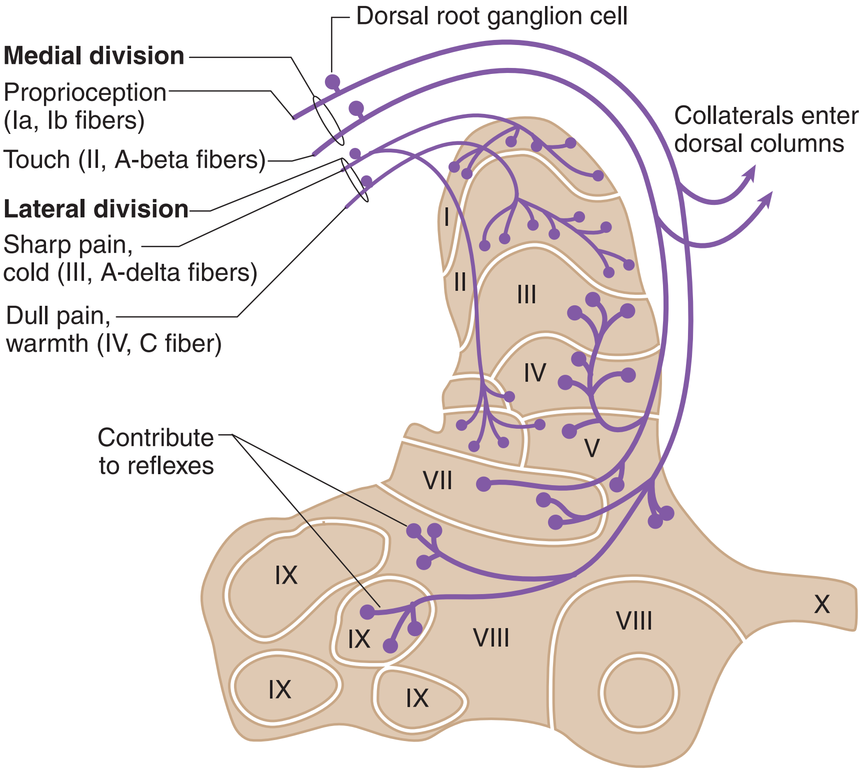


Figure III-4-3. Fiber Types in Dorsal Roots and Sites of Termination in the Spinal Cord Gray Matter

Ventral Horn

The ventral horn contains **alpha and gamma motoneurons**. The alpha motoneurons innervate skeletal muscle (extrafusal fibers) by way of a specialized synapse at a neuromuscular junction, and the gamma motoneurons innervate the contractile intrafusal muscle fibers of the muscle spindle. Within the ventral horn, alpha and gamma motoneurons that innervate flexors are dorsal to those that innervate extensors. Alpha and gamma motoneurons that innervate the proximal musculature are medial to those that innervate the distal musculature. Axons of alpha and gamma motoneurons and axons of preganglionic autonomic neurons leave the cord by way of a ventral root.

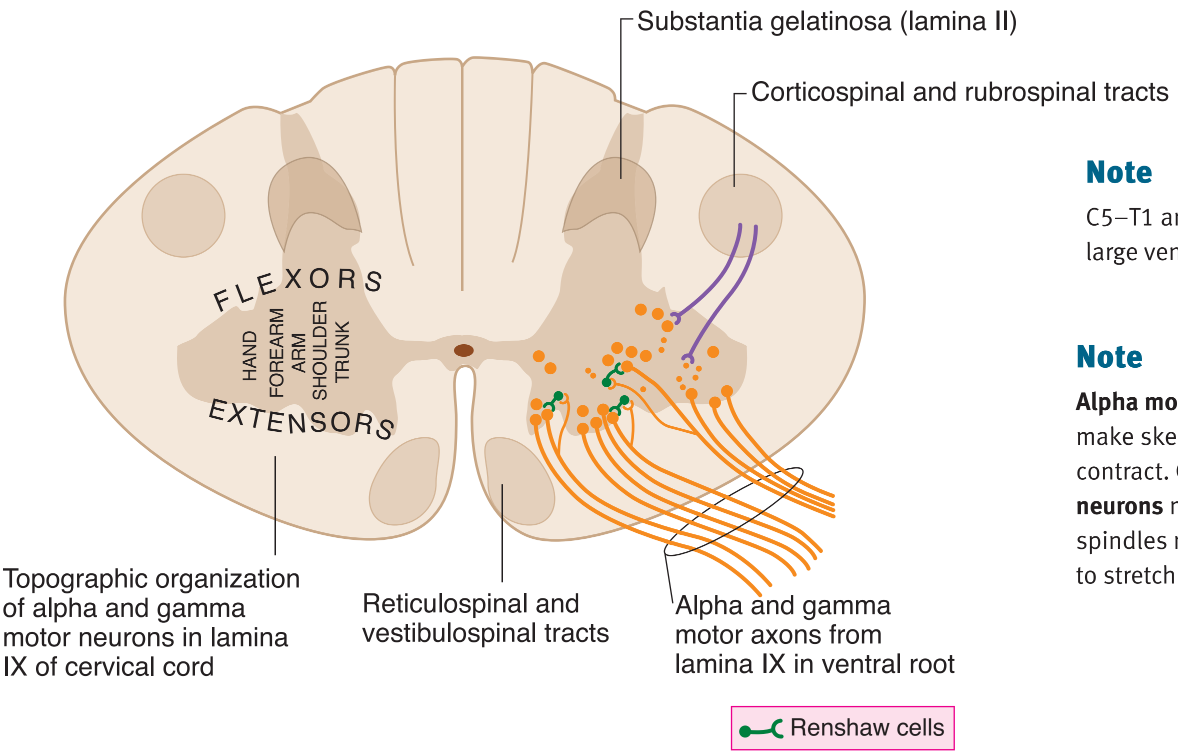


Figure III-4-4. Topographic Organization of Alpha and Gamma Motoneurons (LMNs) in Lamina IX

Note

C5–T1 and L2–S3 have large ventral horn.

Note

Alpha motor neurons make skeletal muscles contract. **Gamma motor neurons** make muscle spindles more sensitive to stretch.

Intermediate Zone

The intermediate zone of the spinal cord from T1 to L2 contains preganglionic sympathetic neuron cell bodies and Clarke nucleus, which sends unconscious proprioception to the cerebellum.

NEURAL SYSTEMS

There are 3 major neural systems in the spinal cord that use neurons in the gray matter and tracts or fasciculi of axons in the white matter. These neural systems have components which can be found at all levels of the CNS, from the cerebral cortex to the tip of the spinal cord. A knowledge of these 3 neural systems is essential to understanding the effects of lesions in the spinal cord and brain stem, and at higher levels of the CNS.

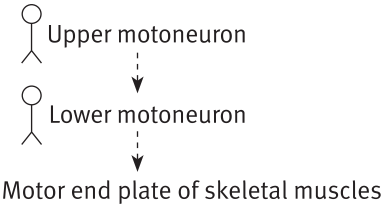
Motor System

Voluntary innervation of skeletal muscle

Upper and Lower Motoneurons

Two motoneurons, an upper motoneuron and a lower motoneuron, together form the basic neural circuit involved in the voluntary contraction of skeletal muscle everywhere in the body. **The lower motoneurons are found in the ventral horn of the spinal cord** and in **cranial nerve nuclei in the brain stem.**

Note



Axons of lower motoneurons of spinal nerves exit in a ventral root, then join the spinal nerve to course in one of its branches to reach and synapse directly at a neuromuscular junction in skeletal muscle. Axons of lower motoneurons in the brain stem exit in a cranial nerve.

To initiate a voluntary contraction of skeletal muscle, a lower motoneuron must be innervated by an upper motoneuron. The **cell bodies of upper motoneurons are found in the brain stem and cerebral cortex**, and their axons descend into the spinal cord in a tract to reach and synapse on lower motoneurons, or on interneurons, which then synapse on lower motoneurons. At a minimum, therefore, to initiate a voluntary contraction of skeletal muscle, 2 motoneurons, an upper and a lower, must be involved. The upper motoneuron innervates the lower motoneuron, and the lower motoneuron innervates the skeletal muscle.

The cell bodies of upper motoneurons are found in the red nucleus, reticular formation, and lateral vestibular nuclei of the brain stem, but the **most important location of upper motoneurons** is in the **cerebral cortex**. Axons of these cortical neurons course in the **corticospinal tract**.

Note

UMNs have net inhibitory effect on muscle stretch reflexes.

Note

Voluntary contraction: UMN → LMN

Reflect contraction: muscle sensory neuron → LMN

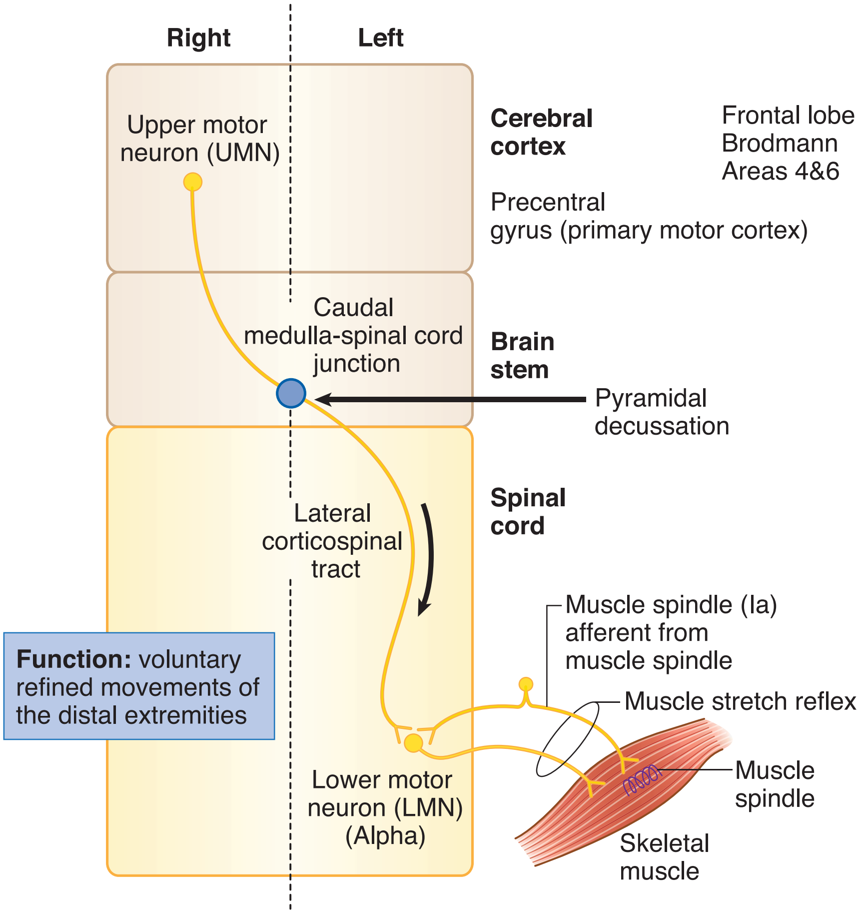


Figure III-4-5. Voluntary Contraction of Skeletal Muscle: UMN and LMNs

Corticospinal Tract

The primary motor cortex, located in the precentral gyrus of the frontal lobe, and the premotor area, located immediately anterior to the primary motor cortex, give rise to about 60% of the fibers of the corticospinal tract (Figure III-4-4). Primary and secondary somatosensory cortical areas located in the parietal lobe give rise to about 40% of the fibers of the corticospinal tract.

Fibers in the corticospinal tract leave the cerebral cortex in the internal capsule, which carries all axons in and out of the cortex. Corticospinal fibers then descend through the length of the brain stem in the ventral portion of the midbrain, pons, and medulla.

In the lower medulla, 80–90% of corticospinal fibers cross at the decussation of the pyramids and continue in the contralateral spinal cord as the lateral corticospinal tract. The lateral corticospinal tract descends the full length of the cord in the lateral part of the white matter. As it descends, axons leave the tract and enter the gray matter of the ventral horn to synapse on lower motoneurons.

Clinical Correlate

Lesions of the Corticospinal Tract

The crossing or decussation of axons of the corticospinal tract at the medulla/spinal cord junction has significant clinical implications. If lesions of the corticospinal tract occur above the pyramidal decussation, a weakness is seen in muscles on the contralateral side of the body; lesions below this level produce an ipsilateral muscle weakness. In contrast to upper motoneurons, the cell bodies of lower motoneurons are ipsilateral to the skeletal muscles that their axons innervate. A lesion to any part of a lower motoneuron will result in an ipsilateral muscle weakness at the level of the lesion.

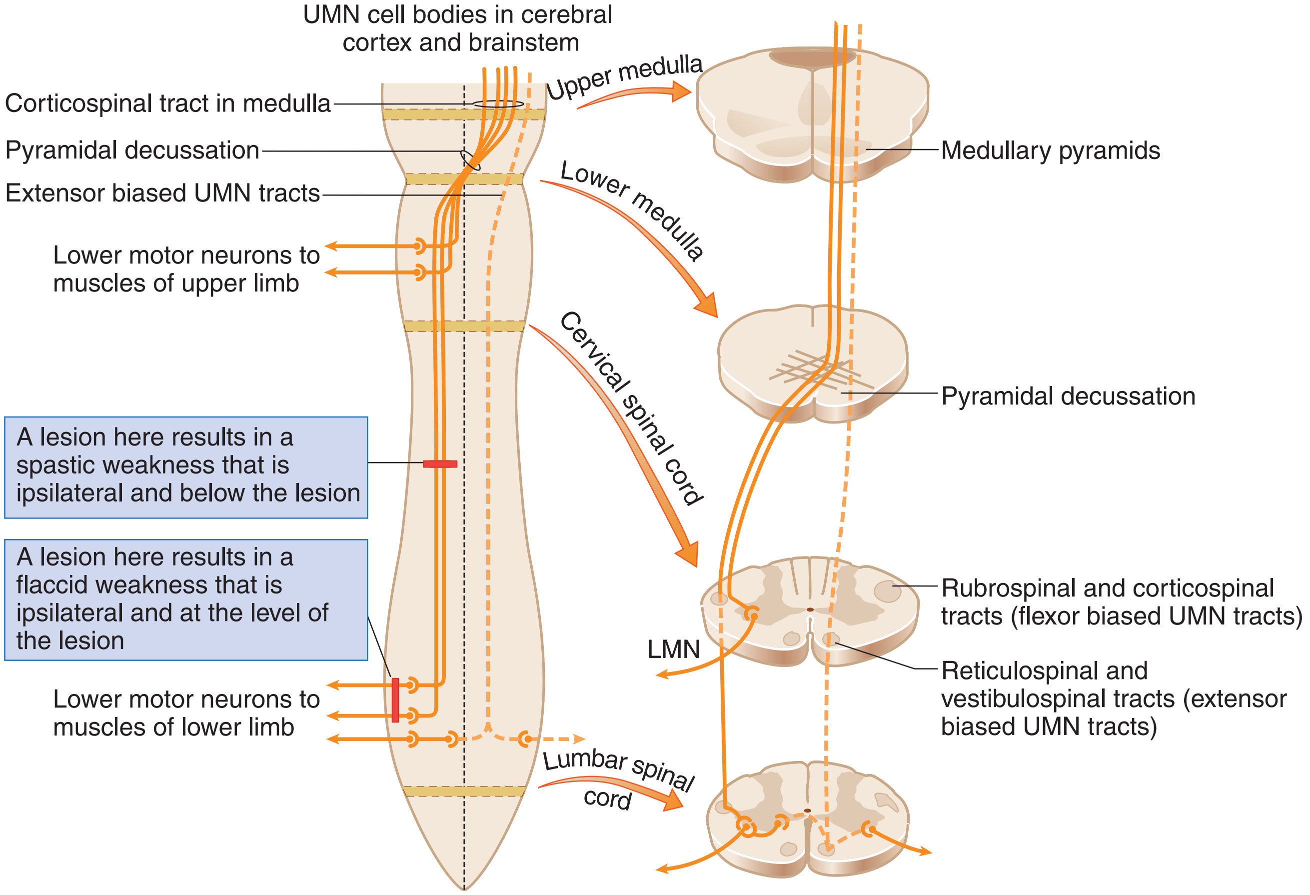


Figure III-4-6. Course of Axons of Upper Motor Neurons in the Medulla and Spinal Cord with Representative Cross-Sections

Reflex innervation of skeletal muscle

A reflex is initiated by a stimulus of a sensory neuron, which in turn innervates a motoneuron and produces a motor response. In reflexes involving skeletal muscles, the sensory stimulus arises from receptors in the muscle, and the motor response is a contraction or relaxation of one or more skeletal muscles. In the spinal cord, lower motoneurons form the specific motor component of skeletal muscle reflexes. **Upper motoneurons provide descending control over the reflexes.**

Both alpha and gamma motoneurons are lower motoneurons that participate in reflexes.

- Alpha motoneurons are large cells in the ventral horn that innervate extrafusal muscle fibers. A single alpha motoneuron innervates a group of muscle fibers, which constitutes a motor unit, the basic unit for voluntary, postural, and reflex activity.
- Gamma motoneurons supply intrafusal muscle fibers, which are modified skeletal muscle fibers. The intrafusal muscle fibers form the muscle spindle, which acts as a sensory receptor in skeletal muscle stretch reflexes.

Both ends of the muscle spindle are connected in parallel with the extrafusal fibers, so these receptors monitor the length and rate of change in length of extrafusal fibers. Muscles involved with fine movements contain a greater density of spindles than those used in coarse movements.

Commonly tested muscle stretch reflexes

The **deep tendon (stretch, myotatic) reflex** is monosynaptic and ipsilateral. The **afferent limb** consists of a **muscle spindle, Ia sensory neuron,** and **efferent limb (lower motor neuron)**. These reflexes are useful in the clinical exam.

Reflex	Cord Segment Involved	Muscle Tested
Knee (patellar)	L2–L4 (femoral n.)	Quadriceps
Ankle	S1 (tibial n.)	Gastrocnemius
Elbow	C5–C6 (musculocutaneous n.)	Biceps
Elbow	C7–C8 (radial n.)	Triceps
Forearm	C5–C6 (radial n.)	Brachioradialis

Muscle stretch (myotatic) reflex

The muscle stretch (myotatic) reflex is the stereotyped contraction of a muscle in response to stretch of that muscle. **The stretch reflex is a basic reflex that occurs in all muscles and is the primary mechanism for regulating muscle tone.** Muscle tone is the tension present in all resting muscle. Tension is controlled by the stretch reflexes.

The best example of a muscle stretch or deep tendon reflex is the knee-jerk reflex. Tapping the patellar ligament stretches the quadriceps muscle and its muscle spindles. Stretch of the spindles activates sensory endings (Ia afferents),

and afferent impulses are transmitted to the cord. Some impulses from stretch receptors carried by Ia fibers monosynaptically stimulate the alpha motoneurons that supply the quadriceps. This causes contraction of the muscle and a sudden extension of the leg at the knee. Afferent impulses simultaneously inhibit antagonist muscles through interneurons (in this case, hamstrings).

Inverse muscle stretch reflex

The inverse muscle stretch reflex monitors muscle tension. This reflex uses Golgi tendon organs (GTOs). These are encapsulated groups of nerve endings that terminate between collagenous tendon fibers at the junction of muscle and tendon. GTOs are oriented in series with the extrafusal fibers and respond to increases in force or tension generated in that muscle. Increases in force in a muscle increase the firing rate of Ib afferent neurons that innervate the GTOs, which, in turn, polysynaptically facilitate antagonists and inhibit agonist muscles.

Muscle tone and reflex activity can be influenced by **gamma motoneurons** and by upper motoneurons. Gamma motoneurons directly innervate the muscle spindles and regulate their sensitivity to stretch. Upper motoneurons innervate gamma motoneurons and also influence the sensitivity of muscle spindles to stretch. Stimulation of gamma motoneurons causes intrafusal muscle fibers located at the pole of each muscle spindle to contract, which activates alpha motoneurons, causing an increase in muscle tone.

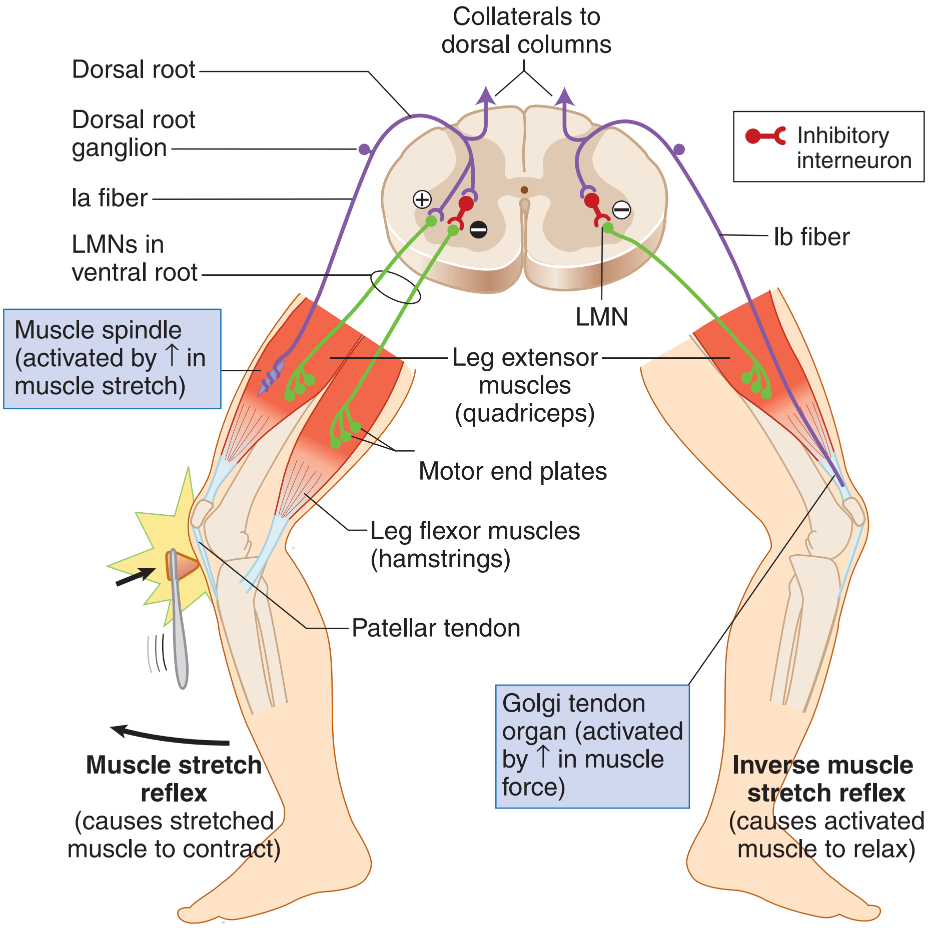


Figure III-4-7. Muscle Stretch and Golgi Tendon Reflex Components

Note

UMN lesions result in:

- Hyperactive muscle stretch reflexes
- Clasp knife reflex due to oversensitive Golgi tendon organs

Flexor withdrawal reflex

The flexion withdrawal reflex is a protective reflex in which a stimulus (usually painful) causes withdrawal of the stimulated limb. This reflex may be accompanied by a crossed extension reflex in which the contralateral limb is extended to help support the body.

Clinical Correlate

Upper Motoneuron Versus Lower Motoneuron Muscle Lesions

A fundamental requirement of interpreting the cause of motor weakness in neuroscience cases is the ability to distinguish between a lesion of an upper versus a lower motoneuron. Because a lesion to either one produces a weakness in the ability to voluntarily contract skeletal muscles, the key to distinguishing them will be the condition of reflexes of the affected muscles.

A lesion of any part of a lower motoneuron will result in hypoactive muscle stretch reflexes and a reduction in muscle tone (hypotonicity) because lower motoneurons form the motor component of the reflex. Therefore, lower motoneuron lesions result in a paresis combined with suppressed or absent muscle stretch reflexes. An early sign is muscle fasciculations, which are twitches or contractions of groups of muscle fibers, that may produce a twitch visible on the skin. Later, lower motoneuron lesions produce fibrillations, which are invisible 1- to 5-ms potentials, detected with electromyography. Muscles denervated by a lower motoneuron lesion undergo pronounced wasting or atrophy. The constellation of signs combining paresis with suppressed or absent reflexes, fasciculations, and atrophy is known as a **flaccid paralysis.** With few exceptions, lower motoneuron lesions produce a flaccid paralysis ipsilateral and at the level of the lesion.

Neurologically, upper motoneurons including the corticospinal tract have a net overall inhibitory effect on muscle stretch reflexes. As a result, they combine paresis of skeletal muscles with muscle stretch or deep tendon reflexes that are hyperactive or hypertonic. The hypertonia may be seen as decorticate rigidity (i.e., postural flexion of the arm and extension of the leg) or decerebrate rigidity (i.e., postural extension of the arm and leg) depending on the location of the lesion. Lesions above the midbrain produce decorticate rigidity; lesions below the midbrain produce decerebrate rigidity. Upper motoneuron lesions result in atrophy of weakened muscles only as a result of disuse, because these muscles can still be contracted by stimulating muscle stretch reflexes.

Upper motoneuron lesions are also accompanied by reversal of cutaneous reflexes, which normally yield a flexor motor response. The best known of the altered flexor reflexes is the **Babinski sign.** The test for the Babinski reflex is performed by stroking the lateral surface of the sole of the foot with a slightly painful stimulus. Normally, there is plantar flexion of the big toe. With a lesion of the corticospinal tract, the Babinski reflex is present, which is characterized by extension of the great toe and fanning of the other toes. Two other flexor reflexes, the abdominal and cremasteric, are also lost in upper motoneuron lesions. The constellation of signs combining paresis with increases or hyperactive reflexes, disuse atrophy of skeletal muscles, and altered cutaneous reflexes is known as a spastic paresis.

In contrast to lower motoneuron lesions, lesions of upper motoneurons result in a **spastic paresis** that is ipsilateral or contralateral and below the site of the lesion.
Upper motoneuron lesions anywhere in the spinal cord will result in an ipsilateral spastic paresis below the level of the lesion. Upper motoneuron lesions between the cerebral cortex and the medulla above the decussation of the pyramids will result in a contralateral spastic paresis below the level of the lesion.

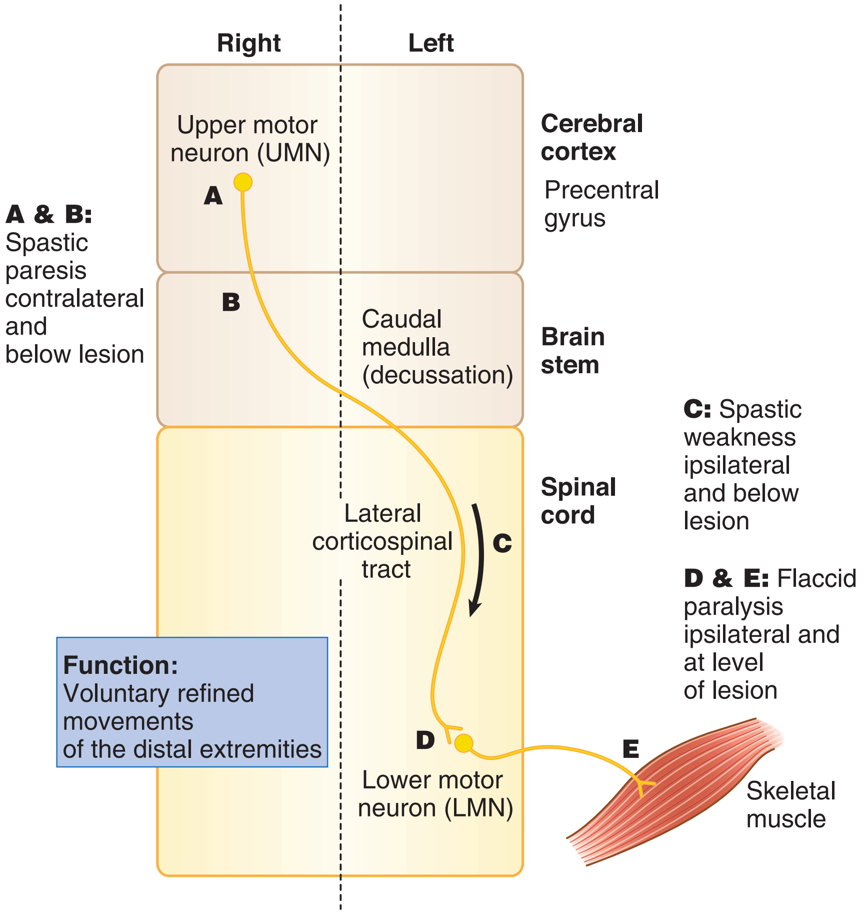


Figure III-4-8. Upper Versus Lower Motor Neuron Lesions

Table III-4-2. Upper Versus Lower Motoneuron Lesions

Upper Motor Neuron Lesion*	Lower Motor Neuron Lesion†
Spastic paresis	Flaccid paralysis
Hyperreflexia	Areflexia
Babinski sign present	No Babinski
Increased muscle tone	Fasciculations
Clasp knife reflex	Decreased muscle tone or atonia
Disuse atrophy of muscles	Atrophy of muscle(s)
Decreased speed of voluntary movements	Loss of voluntary movements
Large area of the body involved	Small area of the body affected

*Deficits contralateral or ipsilateral and below the lesion
†Deficits ipsilateral and at the level of lesion

Sensory Systems

Two sensory systems, the dorsal column–medial lemniscal system and the anterolateral (spinothalamic) system, use 3 neurons to convey sensory information from peripheral sensory receptors to conscious levels of cerebral cortex. In both systems, the first sensory neuron that innervates a sensory receptor has a cell body in the dorsal root ganglion and carries the information into the spinal cord in the dorsal root of a spinal nerve. The first neuron synapses with a second neuron in the brain stem or the spinal cord, and the axon of the second neuron crosses the midline and is carried in a tract in the CNS. The axon of the second neuron then synapses on a third neuron that is in the thalamus. The axon of the third neuron projects to primary somatosensory cortex.

1: first-order neuron in sensory ganglion

2: second-order neuron in CNS (axon always crosses midline)

3: third-order neuron in thalamus

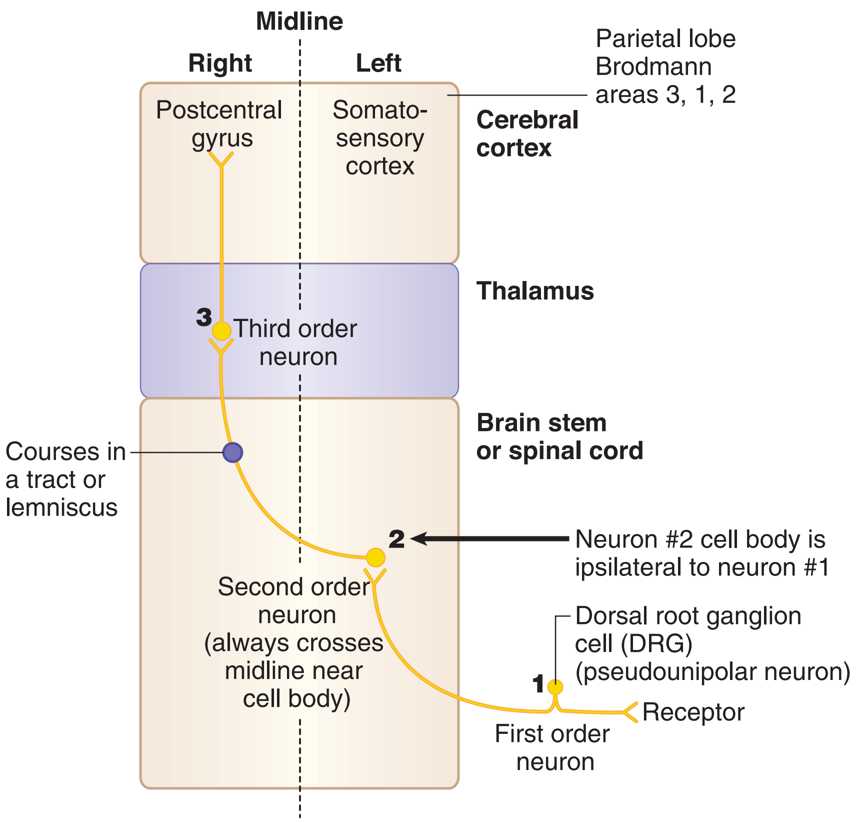


Figure III-4-9. General Sensory Pathways

Ascending Pathways

The 2 most important ascending pathways use a 3-neuron system to convey sensory information to the cortex. Key general features are listed below.

Pathway	Function	Overview
Dorsal column–medial lemniscal system	Discriminative touch, conscious proprioception, vibration, pressure	**3-neuron system:** 1° neuron: cell body in **DRG** 2° neuron: **decussates** 3° neuron: **thalamus (VPL) → cortex**
Anterolateral system (spinothalamic)	Pain and temperature	

Abbreviations: DRG, dorsal root ganglia; VPL, ventral posterolateral nucleus.

Dorsal column–medial lemniscal system

The dorsal column–medial lemniscal system carries sensory information for discriminative touch, joint position (kinesthetic or conscious proprioceptive) sense, vibratory, and pressure sensations from the trunk and limbs. The primary afferent neurons in this system have their cell bodies in the dorsal root ganglia, enter the cord via class II or A-beta dorsal root fibers, and then coalesce in the fasciculus gracilis or fasciculus cuneatus in the dorsal funiculus of the spinal cordfasciculus cuneatus in the dorsal funiculus of the spinal cord. The fasciculus gracilis, found at all spinal cord levels, is situated closest to the midline and carries input from the lower extremities and lower trunk. The fasciculus cuneatus, found only at upper thoracic and cervical spinal cord levels, is lateral to the fasciculus gracilis and carries input from the upper extremities and upper trunk. These 2 fasciculi form the dorsal columns of the spinal cord that carry the central processes of dorsal root ganglion cells and ascend the length of the spinal cord to reach their second neurons in the lower part of the medulla.

In the lower part of the medulla, fibers in the fasciculus gracilis and fasciculus cuneatus synapse with the second neurons found in the nucleus gracilis and nucleus cuneatus, respectively. Cells in these medullary nuclei give rise to fibers that cross the midline as internal arcuate fibers and ascend through the brain stem in the medial lemniscus ascend through the brain stem in the medial lemniscus. Fibers of the medial lemniscus terminate on cells of the ventral posterolateral (VPL) nucleus of the thalamus. From the VPL nucleus, thalamocortical fibers project to the primary somesthetic (somatosensory) area of the postcentral gyrus, located in the most anterior portion of the parietal lobe.

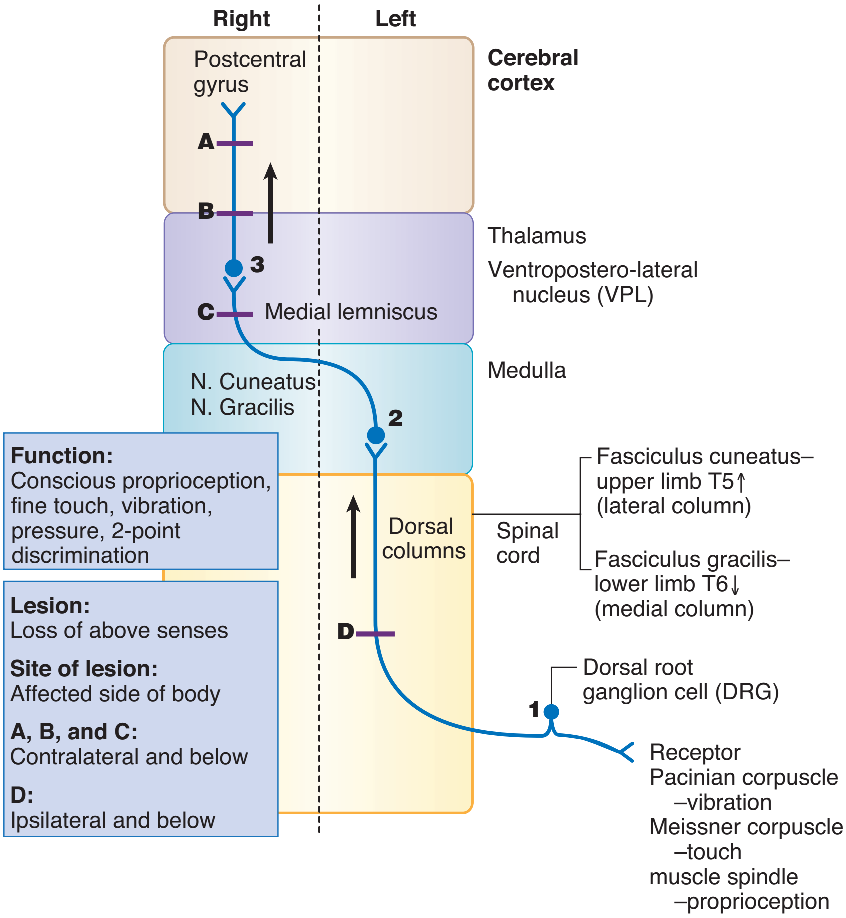


Figure III-4-10. Dorsal Column Pathway–Medial Lemniscal System

Clinical Correlate

Lesions of the dorsal columns result in a loss of joint position sensation, vibratory and pressure sensations, and 2-point discrimination. There is loss of the ability to identify the characteristics of an object, called astereognosis (e.g., size, consistency, form, shape), using only the sense of touch. Typically, dorsal column–medial lemniscal lesions are evaluated by testing vibratory sense using a 128-Hz tuning fork. Romberg sign is also used to distinguish between lesions of the dorsal columns and the midline (vermal area) of the cerebellum.

Romberg sign is tested by asking the patients to place their feet together. If there is a marked deterioration of posture (if the patient sways) with the eyes closed, this is a positive Romberg sign, suggesting that the lesion is in the dorsal columns (or dorsal roots of spinal nerves). With the eyes open, interruption of proprioceptive input carried by the dorsal columns can be compensated for by visual input to the cerebellum. Therefore, if the patient has balance problems and tends to sway with the eyes open, this is indicative of cerebellar damage.

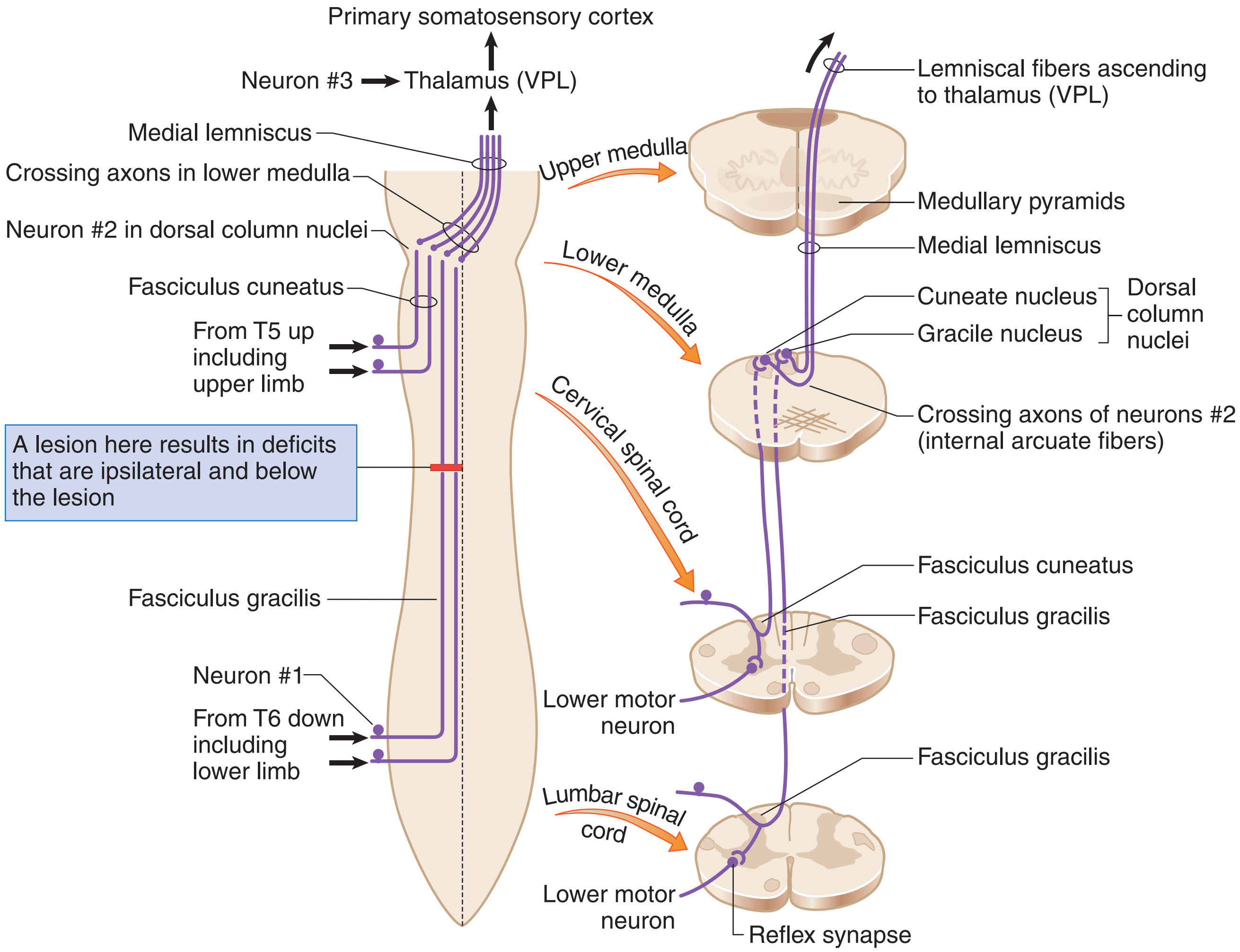


Figure III-4-11. Dorsal Column/Medial Lemniscal System in the Spinal Cord and Medulla

Anterolateral (spinothalamic tract) system

The anterolateral system carries pain, temperature, and crude touch sensations from the extremities and trunk.

Pain and temperature fibers have cell bodies in the dorsal root ganglia and enter the spinal cord via A-delta and C or class III and class IV dorsal root fibers. Their fibers ascend or descend a couple of segments in the dorsolateral tract of Lissauer before entering and synapsing in the dorsal horn. The second neuron cell bodies are located in the dorsal horn gray matter. Axons from these cells cross in the ventral white commissure just below the central canal of the spinal cord and coalesce to form the spinothalamic tract in the ventral part of the lateral funiculus. The spinothalamic tract courses through the entire length of the spinal cord and the brain stem to terminate in the VPL nucleus of the thalamus. Cells in the VPL nucleus send pain and temperature information to the primary somatosensory cortex in the postcentral gyrus.

Clinical Correlate

Because the pain and temperature information crosses almost as soon as it enters the spinal cord, any unilateral **lesion of the spinothalamic tract** in the spinal cord or brain stem will result in a contralateral loss of pain and temperature. This is an extremely useful clinical sign because it means that if a patient presents with analgesia on one side of the trunk or limbs, the location of the lesion must be on the contralateral side of the spinal cord or brain stem. The analgesia begins 1 to 2 segments below the lesion and includes everything below that level.

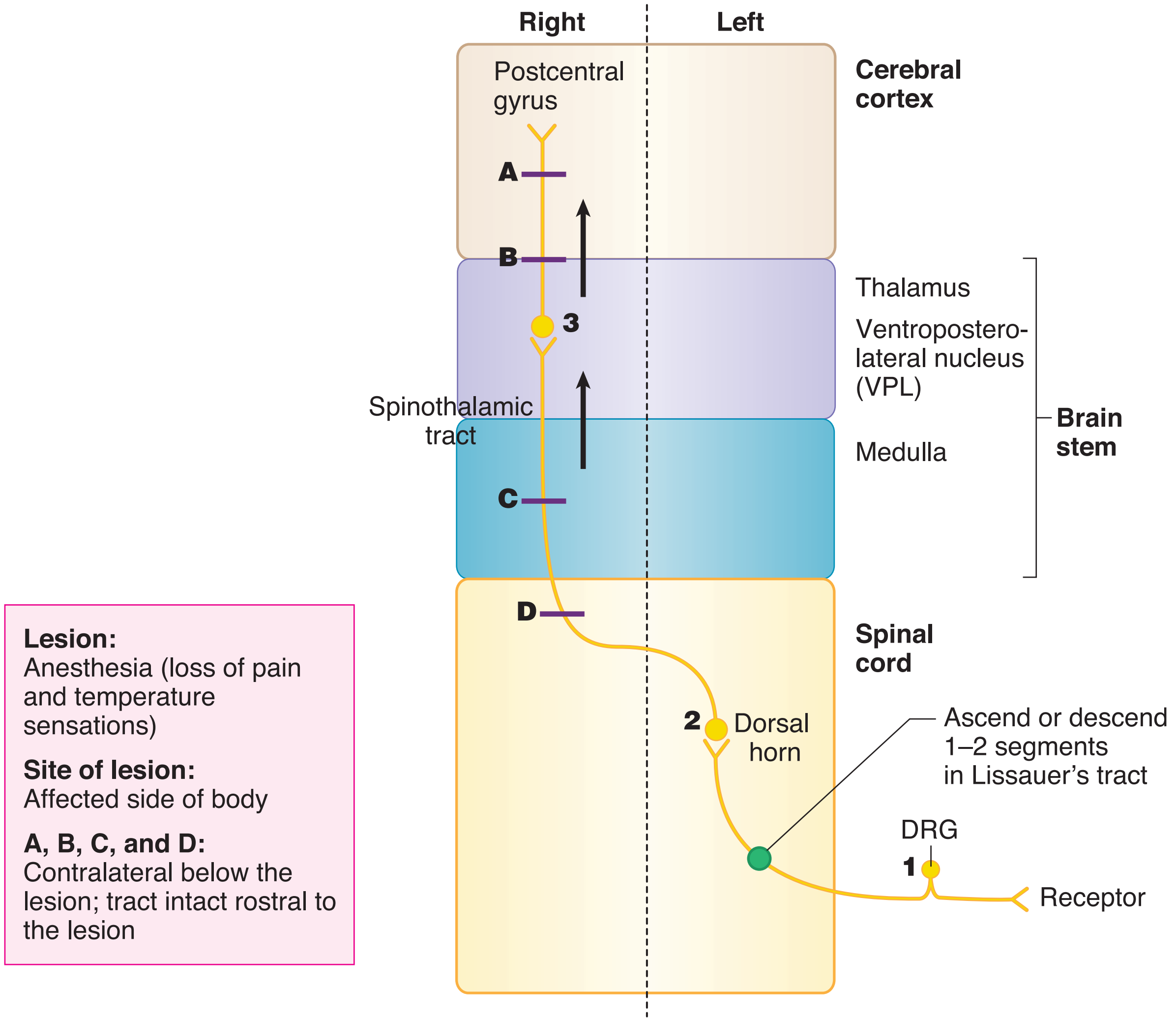


Figure III-4-12. Spinothalamic Tract (Anterolateral System)

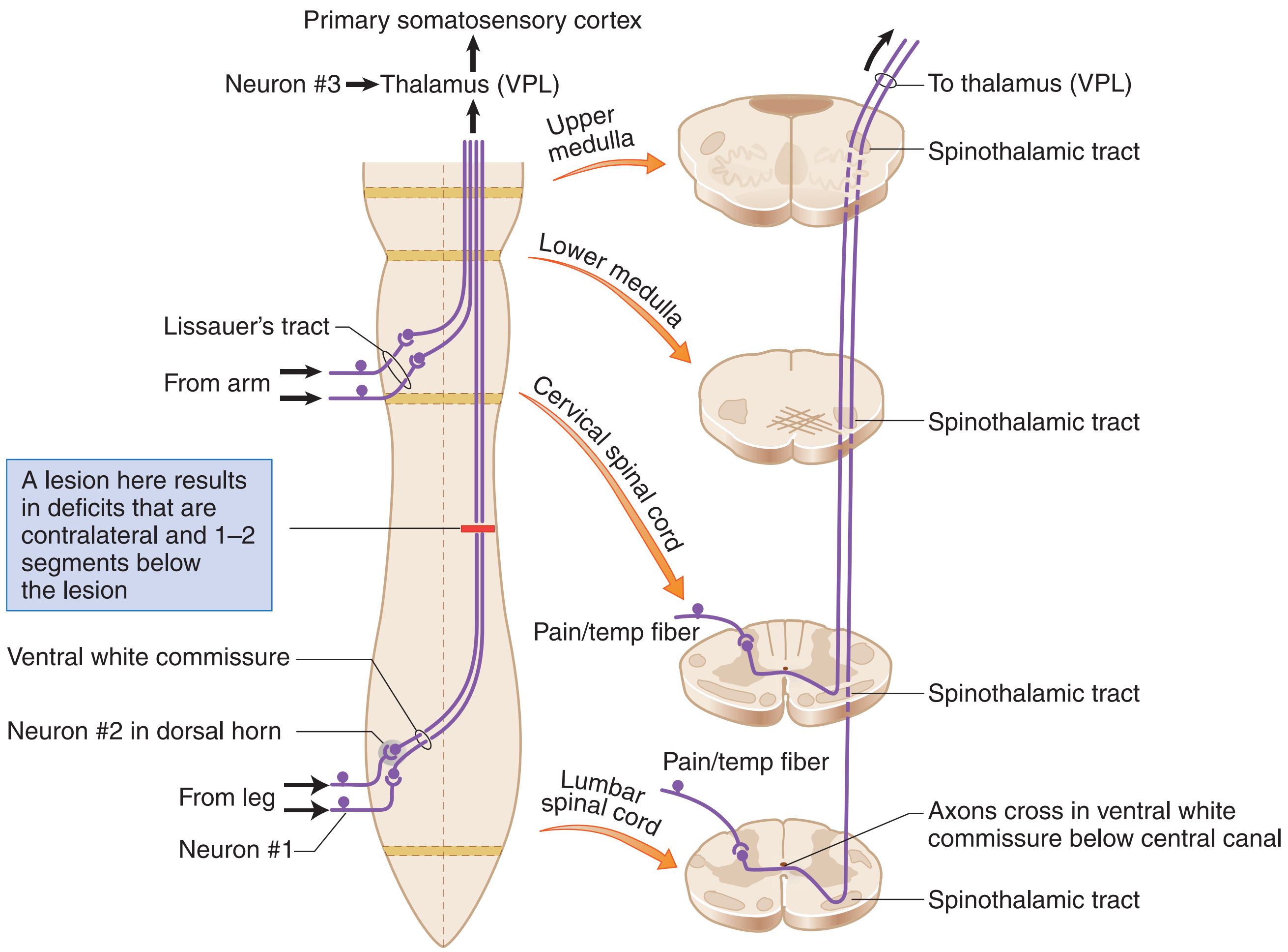


Figure III-4-13. Anterolateral System in the Spinal Cord and Medulla

Spinocerebellar pathways

The spinocerebellar tracts mainly carry unconscious proprioceptive input from muscle spindles and GTOs to the cerebellum, where this information is used to help monitor and modulate movements. There are 2 major spinocerebellar pathways:

- Dorsal spinocerebellar tract—carries input from the lower extremities and lower trunk.
- Cuneocerebellar tract—carries proprioceptive input to the cerebellum from the upper extremities and upper trunk.

The cell bodies of the dorsal spinocerebellar tract are found in Clarke's nucleus, which is situated in the spinal cord from T1 to L2. The cell bodies of the cuneocerebellar tract are found in the medulla in the external cuneate nucleus.

Clinical Correlate

Lesions that affect only the spinocerebellar tracts are uncommon, but there are a group of hereditary diseases in which degeneration of spinocerebellar pathways is a prominent feature. The most common of these is Friedreich ataxia, which is usually inherited as an autosomal recessive trait. The spinocerebellar tracts, dorsal columns, corticospinal tracts, and cerebellum may be involved. Ataxia of gait is the most common initial symptom of this disease.

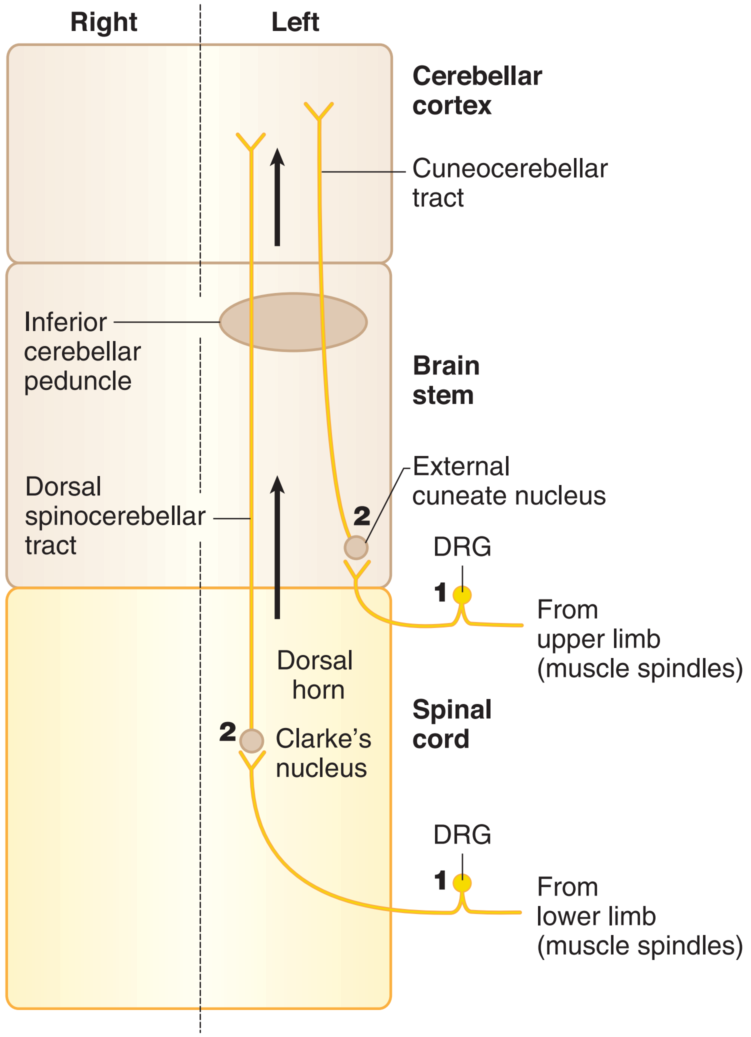


Figure III-4-14. Spinocerebellar Tracts

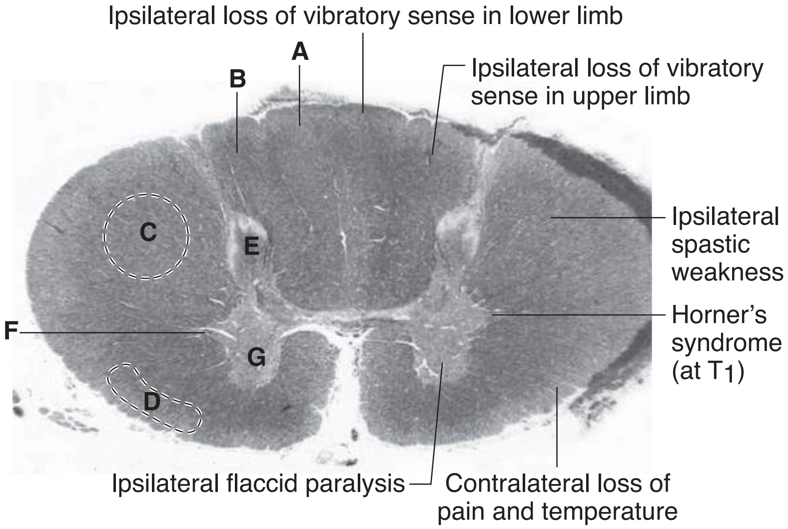


Figure III-4-15. Major spinal cord neural components and clinical anatomy depicted in myelin-stained section of upper thoracic cord.

A Fasciculus Gracilis, B Fasciculus Cuneatus, C Corticospinal tract, D Anterolateral system, E Dorsal Horn, F Lateral horn (preganglionic sympathetic neurons), G Ventral horn (lower motor neurons)

Features to look for to identify a cord section:

- Is there a large ventral horn?
  - Yes → C_5–T_1, or L_2–S_2
  - No → T_2–L_1, C_1–C_4
- Are both dorsal columns present?
  - Yes → Above T_5
  - No → Below T_5
- Is there a lateral horn present?
  - Yes → T_1–L_2
  - No → C_1–C_8 or L_3–S_5

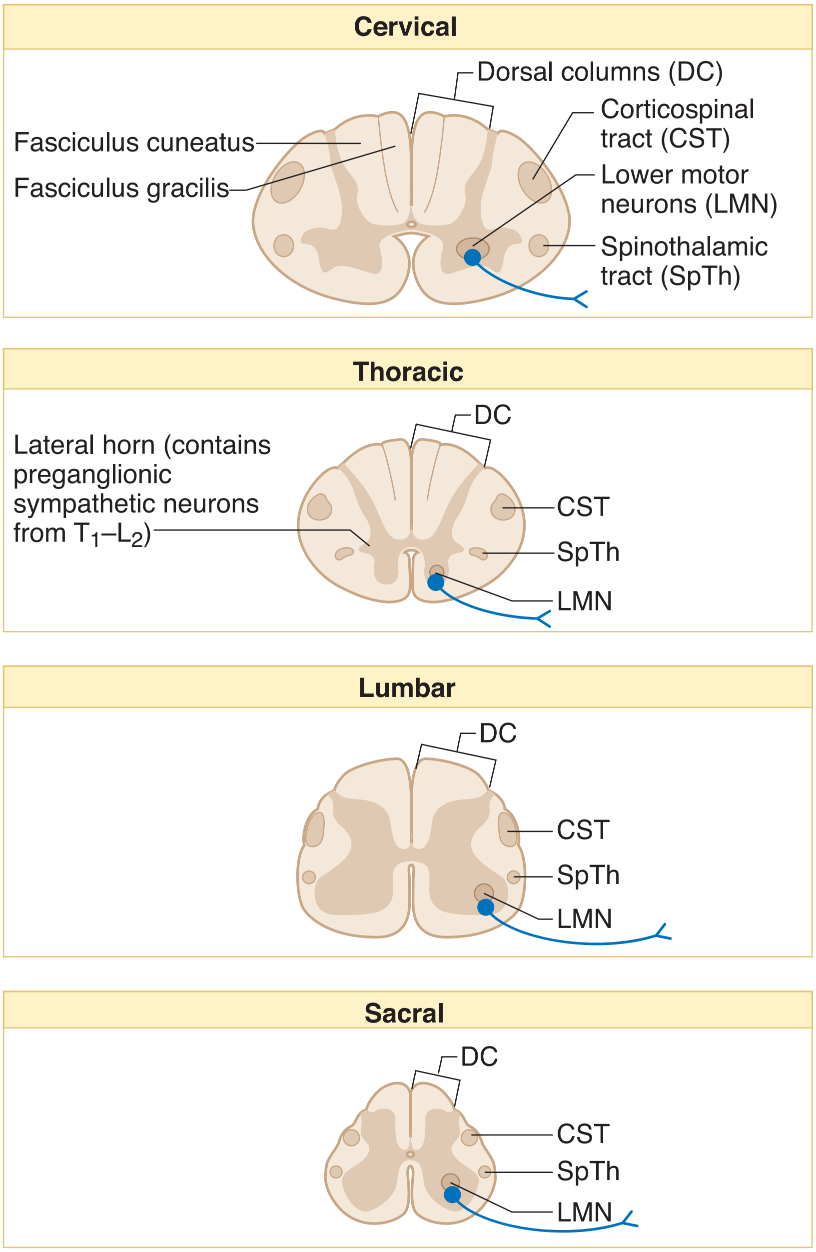


Figure III-4-16. Spinal Cord: Levels

Note

In Argyll Robertson pupil, the pupil reacts to accommodation but not to light.

Spinal Cord Lesions

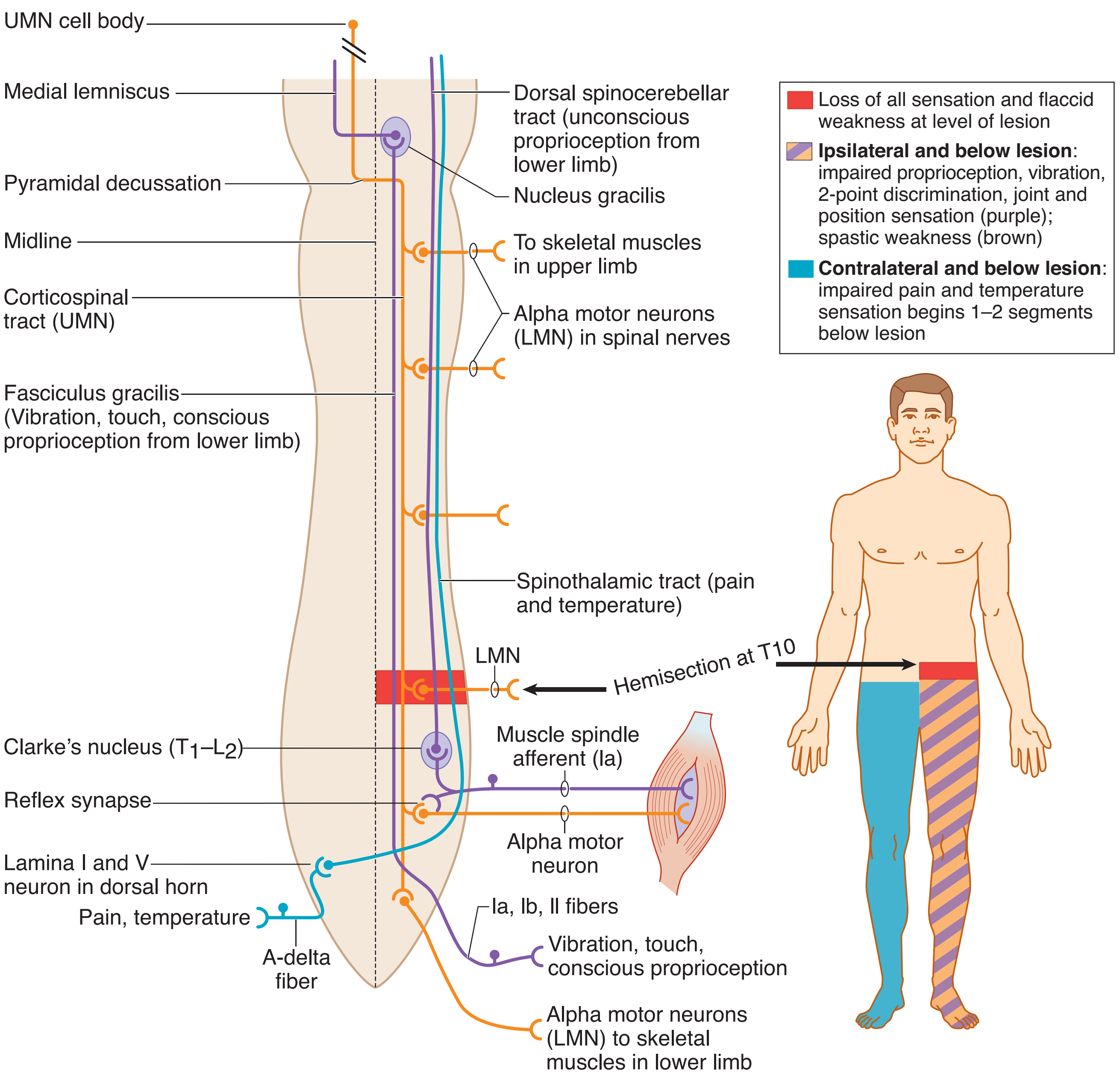


Figure III-4-17. Spinal Cord Overview and Brown-Séquard Syndrome with Lesion at Left Tenth Thoracic Segment

Brown-Séquard syndrome

Hemisection of the cord results in a lesion of each of the 3 main neural systems: the principal upper motoneuron pathway of the corticospinal tract, one or both dorsal columns, and the spinothalamic tract. The hallmark of a lesion to these 3 long tracts is that the patient presents with 2 ipsilateral signs and 1 contralateral sign.

- Lesion of the corticospinal tract results in an ipsilateral spastic paresis below the level of the injury.
- Lesion of the fasciculus gracilis or cuneatus results in an ipsilateral loss of joint position sense, tactile discrimination, and vibratory sensations below the lesion.
- Lesion of the spinothalamic tract results in a contralateral loss of pain and temperature sensation starting 1 or 2 segments below the level of the lesion.

At the level of the lesion, there will be an ipsilateral loss of all sensation, including touch modalities as well as pain and temperature, and an ipsilateral flaccid paralysis in muscles supplied by the injured spinal cord segments (Figure III-4-15).

Clinical Correlate

Tabes patients present with paresthesias (pins-and-needles sensations), pain, polyuria, Romberg sign.

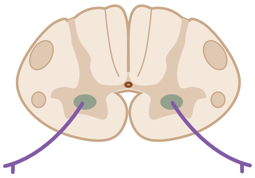
Clinical Correlate

Spastic bladder results from lesions of the spinal cord **above the sacral spinal cord levels**. There is a loss of inhibition of the parasympathetic nerve fibers that innervate the detrusor muscle during the filling stage. Thus, the detrusor muscle responds to a minimum amount of stretch, causing urge incontinence.

Clinical Correlate

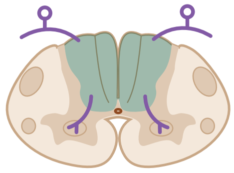
Atonic bladder results from lesions **to the sacral spinal cord segments** or the sacral spinal nerve roots. Loss of pelvic splanchnic motor innervation with loss of contraction of the detrusor muscle results in a full bladder with a continuous dribble of urine from the bladder.

Polio



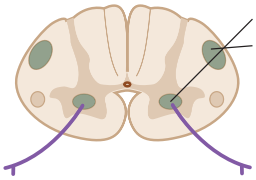
a. Flaccid paralysis
b. Muscle atrophy
c. Fasciculations
d. Areflexia
e. Common at lumbar levels

Tabes Dorsalis



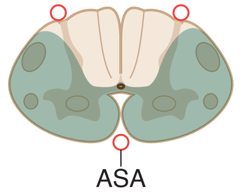
a. “Paresthesias, pain, polyuria”
b. Associated with late-stage syphilis, sensory ataxia, positive Romberg sign: sways with eyes closed, Argyll Robertson pupils, suppressed reflexes
c. Common at lumbar cord levels

Amyotrophic Lateral Sclerosis (ALS)



a. Progressive spinal muscular atrophy (ventral horn)
b. Primary lateral sclerosis (corticospinal tract)
   • Spastic paralysis in lower limbs
   • Increased tone and reflexes
   • Flaccid paralysis in upper limbs
c. Common in cervical enlargement

Anterior Spinal Artery (ASA) Occlusion



a. DC spared
b. All else bilateral signs
c. Common at mid thoracic levels
d. Spastic bladder

Figure III-4-18. Lesions of the Spinal Cord I

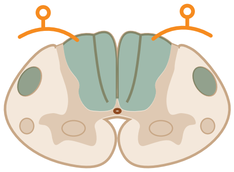
Poliomyelitis

Poliomyelitis results from a relatively selective destruction of lower motoneurons in the ventral horn by the poliovirus. The disease causes a flaccid paralysis of muscles with the accompanying hyporeflexia and hypotonicity. Some patients may recover most function, whereas others progress to muscle atrophy and permanent disability.

Amyotrophic lateral sclerosis

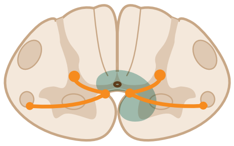
Amyotrophic lateral sclerosis (ALS, Lou Gehrig disease) is a relatively pure motor system disease that affects both upper and lower motoneurons. The disease typically begins at cervical levels of the cord and progresses either up or down the cord. Patients present with bilateral flaccid weakness of the upper limbs and bilateral spastic weakness of the lower limbs. Lower motoneurons in the brain-stem nuclei may be involved later.

Subacute Combined Degeneration



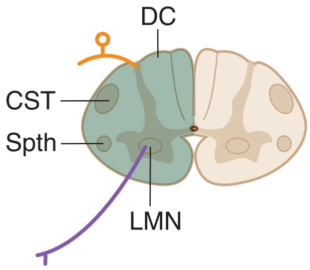
a. Vitamin B12, pernicious anemia
b. Demyelination of the:
  • Dorsal columns (central and peripheral myelin)
  • Spinocerebellar tracts
  • Corticospinal tracts (CST)
c. Upper thoracic or lower cervical cord

Syringomyelia



a. Cavitation of the cord (usually cervical)
b. Bilateral loss of pain and temperature at the level of the lesion
c. As the disease progresses, there is muscle weakness; eventually flaccid paralysis and atrophy of the upper limb muscles due to destruction of ventral horn cells

Hemisection: Brown-Séquard Syndrome (cervical)



a. DC: Ipsilateral loss of position and vibratory senses at and below level of the lesion
b. Spinothalamic tract: Contralateral loss of pain and temp 1–2 segments below lesion and ipsilateral loss at the level of the lesion
c. CST: Ipsilateral paresis below the level of the lesion
d. LMN: Flaccid paralysis at the level of the lesion
e. Descending hypothalamics: Ipsilateral Horner syndrome (if cord lesion is above T1)
  • Facial hemianhydrosis
  • Ptosis (slight)
  • Miosis

Figure III-4-19. Lesions of the Spinal Cord II

Clinical Correlate

Subacute combined degeneration patients present paresthesias, bilateral spastic weakness, Babinski sign Babinski signs, and antibodies to intrinsic factor.

Clinical Correlate

Syringomyelia may present with hydrocephalus and Arnold-Chiari I malformation.

Note

Syringomyelia results in a “belt-like” or “cape-like” loss of pain and temperature.

Occlusion of the anterior spinal artery

This artery lies in the anterior median sulcus of the spinal cord. Occlusion of the anterior spinal artery interrupts blood supply to the ventrolateral parts of the cord, including the corticospinal tracts and spinothalamic tracts. Below the level of the lesion, the patient exhibits a bilateral spastic paresis and a bilateral loss of pain and temperature.

Syringomyelia

Syringomyelia is a disease characterized by progressive cavitation of the central canal, usually in the cervical spinal cord but may involve other cord regions or the medulla. Early in the disease, there is a bilateral loss of pain and temperature sensation in the hands and forearms as a result of the destruction of spinothalamic fibers crossing in the anterior white commissure. When the cavitation expands, lower motoneurons in the ventral horns are compressed, resulting in bilateral flaccid paralysis of upper limb muscles. A late manifestation of cavitation is **Horner syndrome**, which occurs as a result of involvement of descending hypothalamic fibers innervating preganglionic sympathetic neurons in the T1 through T4 cord segments. Horner syndrome consists of miosis (pupillary constriction), ptosis (drooping eyelids), and anhidrosis (lack of sweating) in the face.

Tabes dorsalis

Tabes dorsalis is one possible manifestation of neurosyphilis. It is caused by bilateral degeneration of the dorsal roots and secondary degeneration of the dorsal columns. There may be impaired vibration and position sense, astereognosis, paroxysmal pains, and ataxia, as well as diminished stretch reflexes or incontinence. Owing to the loss of proprioceptive pathways, individuals with tabes dorsalis are unsure of where the ground is and walk with a characteristic and almost diagnostic "high-step stride". Tabetic patients may also present with abnormal pupillary responses (Argyll Robertson pupils).

Subacute combined degeneration

Subacute combined degeneration is seen most commonly in cases of vitamin B12 deficiency, sometimes related to pernicious anemia. The disease is characterized by patchy losses of myelin in the dorsal columns and lateral corticospinal tracts, resulting in a bilateral spastic paresis and a bilateral alteration of touch, vibration, and pressure sensations below the lesion sites. Myelin in both CNS and PNS is affected.

The Brain Stem 5

Learning Objectives

- ❑ Answer questions about cranial nerves
- ❑ Answer questions about sensory and motor neural systems
- ❑ Solve problems concerning medulla
- ❑ Demonstrate understanding of pons
- ❑ Interpret scenarios on midbrain
- ❑ Interpret scenarios on components of the ear, auditory, and vestibular systems
- ❑ Demonstrate understanding of horizontal conjugate gaze
- ❑ Solve problems concerning blood supply to the brain stem
- ❑ Interpret scenarios on brain-stem lesions
- ❑ Interpret scenarios on reticular formation

The brain stem is divisible into 3 continuous parts: the midbrain, the pons, and the medulla. The **midbrain** is most rostral and begins just below the diencephalon. The **pons** is in the middle and is overlain by the cerebellum. The **medulla** is caudal to the pons and is continuous with the spinal cord.

The brain stem is the home of the origins or sites of termination of fibers in 9 of the 12 cranial nerves (CNs).

CRANIAL NERVES

Two cranial nerves, the oculomotor and trochlear (CN III and IV), arise from the midbrain. **Four cranial nerves**, the trigeminal, abducens, facial, and vestibulocochlear nerves (CN V, VI, VII, and VIII), enter or exit from the pons. **Three cranial nerves**, the glossopharyngeal, vagus, and hypoglossal nerves (CN IX, X, and XII), enter or exit from the medulla. Fibers of the accessory nerve arise from the cervical spinal cord.

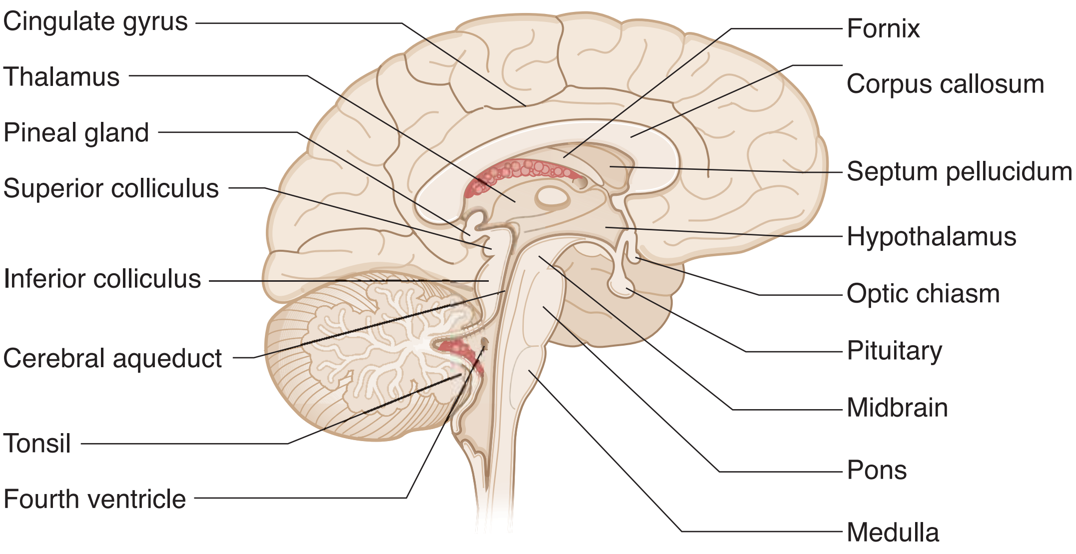


Figure III-5-1. Brain: Mid-Sagittal Section

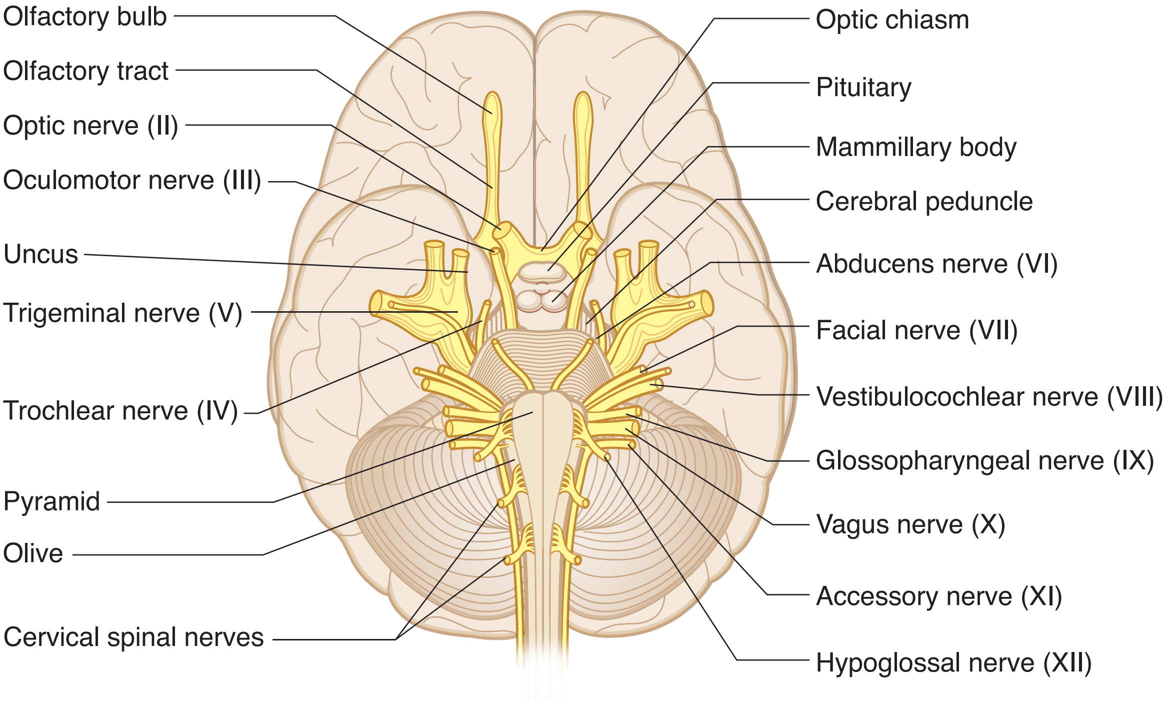


Figure III-5-2. Brain: Inferior View

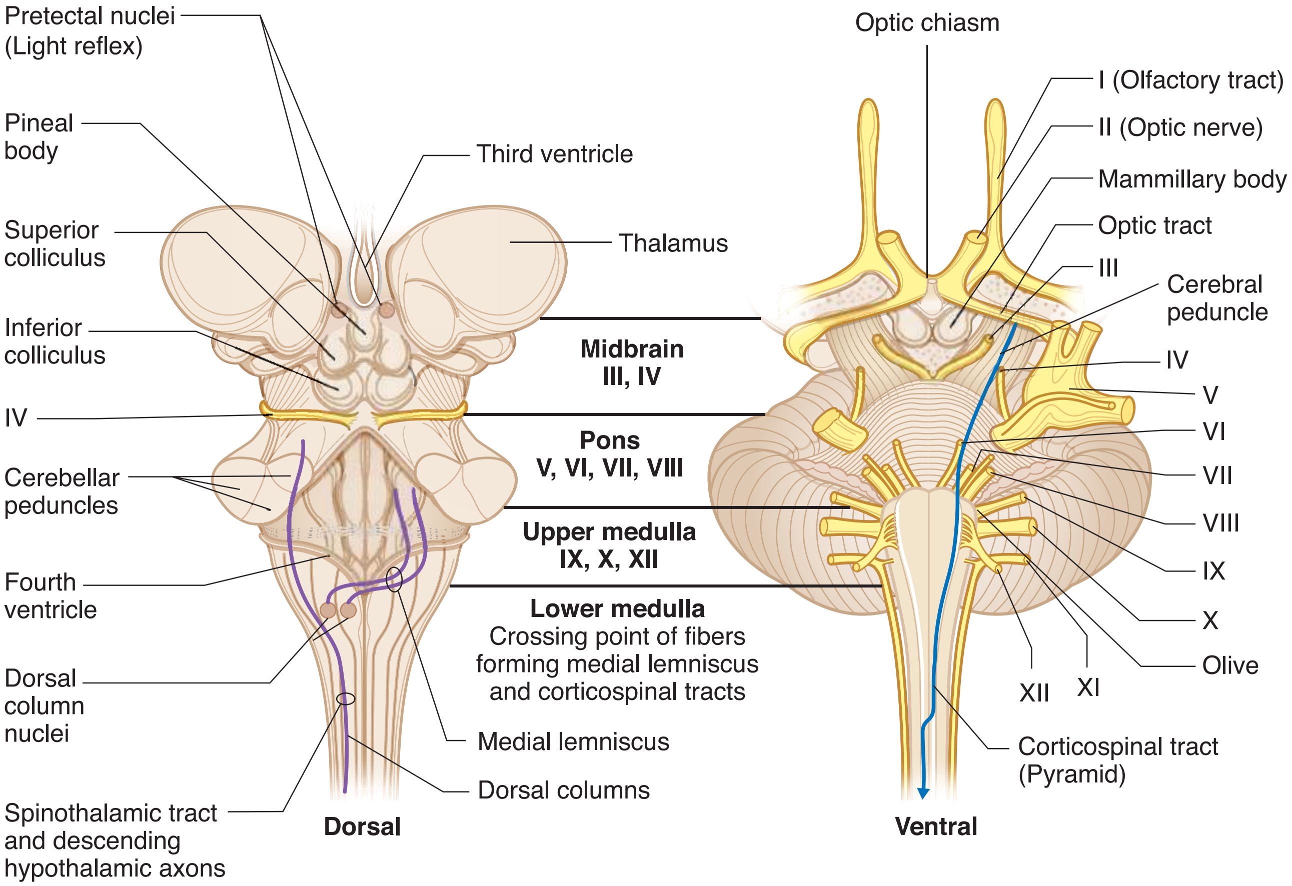


Figure III-5-3. Brain Stem and Cranial Nerve: Surface Anatomy

Afferent fibers of cranial nerves enter the CNS and terminate in relation to aggregates of neurons in sensory nuclei. Motor or efferent components of cranial nerves arise from motor nuclei. All motor and sensory nuclei that contribute fibers to cranial nerves are organized in a series of discontinuous columns according to the functional component that they contain. Motor nuclei are situated medially, closest to the midline, and sensory nuclei are situated lateral to the motor nuclei. A cranial nerve nucleus or nerve will be found at virtually every transverse sectional level of the brain stem.

Note

The descending hypothalamic fibers course with the spinothalamic tract.

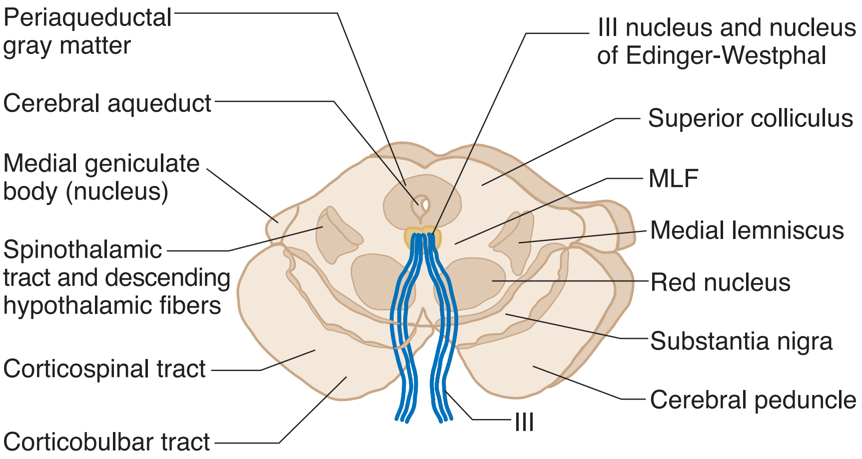


Figure III-5-4A. Upper Midbrain; Level of Nerve III

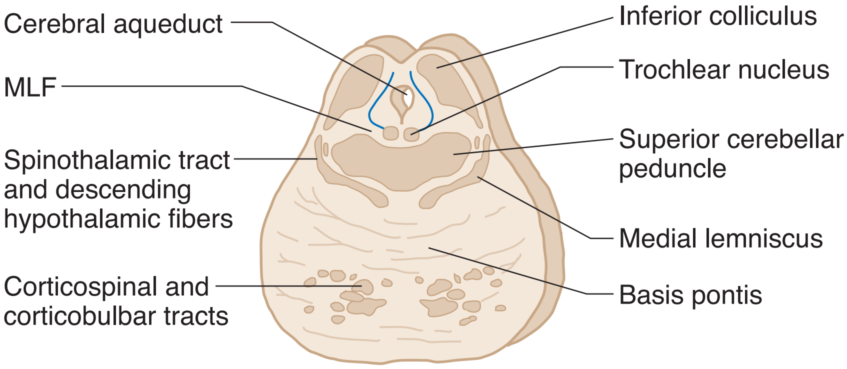


Figure III-5-4B. Lower Midbrain; Level of Nucleus CN IV

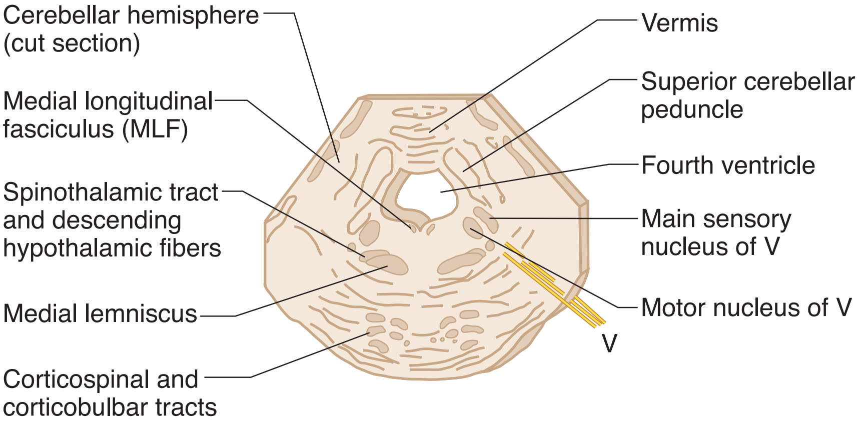


Figure III-5-4C. Middle Pons; Level of Nerve V

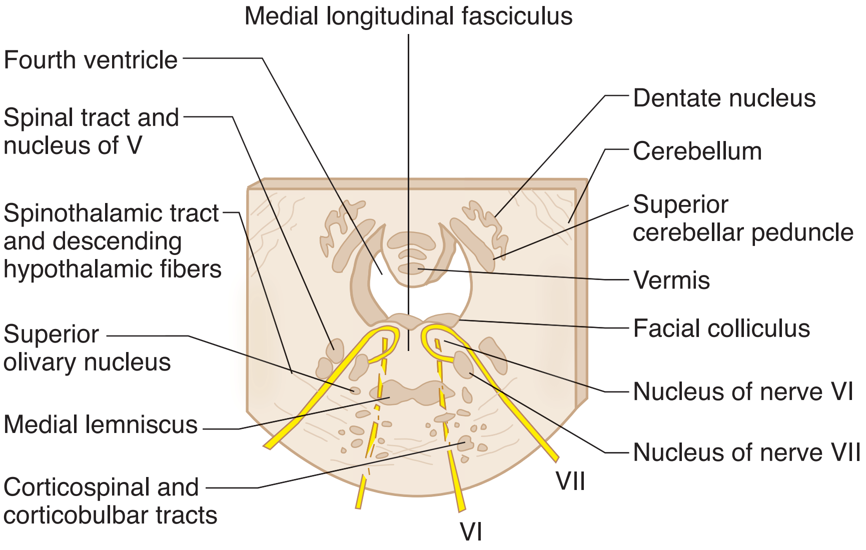


Figure III-5-4D. Lower Pons; Level of Nerves VI and VII

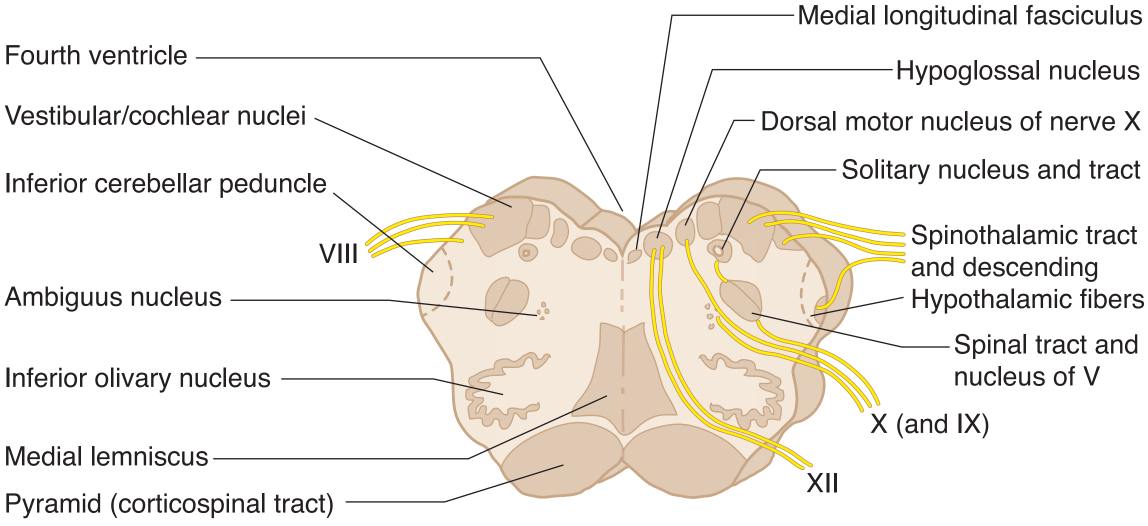


Figure III-5-4E. Open Medulla

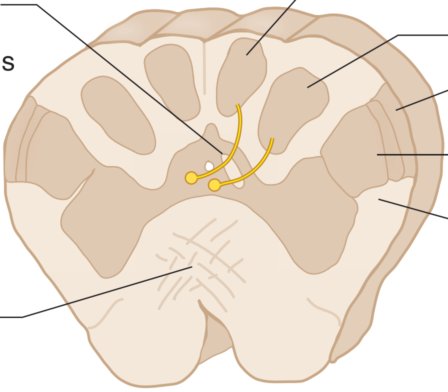


Figure III-5-4F. Closed Medulla

Table III-5-1. Cranial Nerves: Functional Features

CN	Name	Type	Function	Results of Lesions
I	Olfactory	Sensory	Smells	Anosmia
II	Optic	Sensory	Sees	Visual field deficits (anopsia) Loss of light reflex with III Only nerve to be affected by MS
III	Oculomotor	Motor	Innervates SR, IR, MR, IO extraocular muscles: adduction (MR) most important action Raises eyelid (levator palpebrae superioris) Constricts pupil (sphincter pupillae) Accommodates (ciliary muscle)	Diplopia, external strabismus Loss of parallel gaze Ptosis Dilated pupil, loss of light reflex with II Loss of near response
CN	**Name**	**Type**	**Function**	**Results of Lesions**
IV	Trochlear	Motor	Superior oblique—depresses and abducts eyeball (makes eyeball look down and out) Intorts	Weakness looking down with adducted eye Trouble going down stairs Head tilts away from lesioned side
V	Trigeminal Ophthalmic (V1) Maxillary (V2) Mandibular (V3)	Mixed	General sensation (touch, pain, temperature) of forehead/scalp/cornea General sensation of palate, nasal cavity, maxillary face, maxillary teeth General sensation of anterior two-thirds of tongue, mandibular face, mandibular teeth Motor to muscles of mastication (temporalis, masseter, medial and lateral pterygoids) and anterior belly of digastric, mylohyoid, tensor tympani, tensor palati	V1—loss of general sensation in skin of forehead/scalp Loss of blink reflex with VII V2—loss of general sensation in skin over maxilla, maxillary teeth V3—loss of general sensation in skin over mandible, mandibular teeth, tongue, weakness in chewing Jaw deviation toward weak side Trigeminal neuralgia—intractable pain in V2 or V3 territory

Table III-5-1. Cranial Nerves: Functional Features (continued)

CN	Name	Type	Function	Results of Lesions
VI	Abducens	Motor	Lateral rectus—abducts eyeball	Diplopia, internal strabismus Loss of parallel gaze, “pseudoptosis”
VII	Facial	Mixed	To muscles of facial expression, posterior belly of digastric, stylohyoid, stapedius Salivation (submandibular, sublingual glands) Skin behind ear Taste in anterior 2/3 of tongue/palate Tears (lacrimal gland)	Corner of mouth droops, cannot close eye, cannot wrinkle forehead, loss of blink reflex, hyperacusis; Bell palsy—lesion of nerve in facial canal Pain behind ear Alteration or loss of taste (ageusia) Eye dry and red
VIII	Vestibulocochlear	Sensory	Hearing Angular acceleration (head turning) Linear acceleration (gravity)	Sensorineural hearing loss Loss of balance, nystagmus
IX	Glossopharyngeal	Mixed	Oropharynx sensation, carotid sinus/body Salivation (parotid gland) All sensation of posterior one-third of tongue Motor to one muscle—stylopharyngeus	Loss of gag reflex with X
X	Vagus	Mixed	To muscles of palate and pharynx for swallowing except tensor palati (V) and stylopharyngeus (IX) To all muscles of larynx (phonates) Sensory of larynx and laryngopharynx Sensory of GI tract To GI tract smooth muscle and glands in foregut and midgut	Nasal speech, nasal regurgitation Dysphagia, palate droop Uvula pointing away from affected side Hoarseness/fixed vocal cord Loss of gag reflex with IX Loss of cough reflex
CN	**Name**	**Type**	**Function**	**Results of Lesions**
XI	Accessory	Motor	Head rotation to opposite side (sternocleidomastoid) Elevates and rotates scapula (trapezius)	Weakness turning chin to opposite side Shoulder droop
XII	Hypoglossal	Motor	Tongue movement (styloglossus, hyoglossus, genioglossus, and intrinsic tongue muscles—palatoglossus is by X)	Tongue pointing toward same (affected) side on protrusion

Abbreviations: CN, cranial nerve; IO, inferior oblique; IR, inferior rectus; MR, medial rectus; MS, multiple sclerosis; SR, superior rectus

SENSORY AND MOTOR NEURAL SYSTEMS

Each of the following 5 ascending or descending neural tracts, fibers, or fasciculi courses through the brain stem and is found at every transverse sectional level.

The **medial lemniscus** (ML) contains the axons from cell bodies found in the dorsal column nuclei (gracilis and cuneatus) in the caudal medulla and represents the second neuron in the pathway to the thalamus and cortex for discriminative touch, vibration, pressure, and conscious proprioception. The axons in the ML cross the midline of the medulla immediately after emerging from the dorsal column nuclei. Lesions in the ML, in any part of the brain stem, result in a loss of discriminative touch, vibration, pressure, and conscious proprioception from the **contralateral** side of the body.

The **spinothalamic tract** (part of anterolateral system) has its cells of origin in the spinal cord and represents the crossed axons of the second neuron in the pathway conveying pain and temperature to the thalamus and cortex. Lesions of the spinothalamic tract, in any part of the brain stem, result in a loss of pain and temperature sensations from the **contralateral** side of the body.

The **corticospinal tract** controls the activity of lower motoneurons, and interneuron pools for lower motoneurons course through the brain stem on their way to the spinal cord. Lesions of this tract produce a spastic paresis in skeletal muscles of the body **contralateral** to the lesion site in the brain stem.

The **descending hypothalamic fibers** arise in the hypothalamus and course without crossing through the brain stem to terminate on preganglionic sympathetic neurons in the spinal cord. Lesions of this pathway produce an ipsilateral **Horner syndrome.** Horner syndrome consists of miosis (pupillary constriction), ptosis (drooping eyelid), and anhidrosis (lack of sweating) in the face ipsilateral to the side of the lesion.

Descending hypothalamic fibers course with the spinothalamic fibers in the lateral part of the brain stem. Therefore, brain stem lesions producing Horner syndrome may also result in a contralateral loss of pain and temperature sensations from the limbs and body.

The **medial longitudinal fasciculus** is a fiber bundle interconnecting centers for horizontal gaze, the vestibular nuclei, and the nerve nuclei of CN III, IV, and VI, which innervate skeletal muscles that move the eyeball. This fiber bundle courses close to the dorsal midline of the brain stem and also contains vestibulospinal fibers, which course through the medulla to the spinal cord. Lesions of the fasciculus produce **internuclear ophthalmoplegia** and disrupt the vestibulo-ocular reflex.

MEDULLA

In the caudal medulla, 2 of the neural systems—the corticospinal and dorsal column–medial lemniscal pathways—send axons across the midline. The nucleus gracilis and nucleus cuneatus give rise to axons that decussate in the caudal medulla (the crossing axons are the internal arcuate fibers), which then form and ascend in the medial lemniscus.

The corticospinal (pyramidal) tracts, which are contained in the pyramids, course ventromedially through the medulla. Most of these fibers decussate in the caudal medulla just below the crossing of axons from the dorsal column nuclei, and then travel down the spinal cord as the (lateral) corticospinal tract.

The olives are located lateral to the pyramids in the rostral two-thirds of the medulla. The olives contain the convoluted inferior olivary nuclei. The olivary nuclei send climbing (olivocerebellar) fibers into the cerebellum through the inferior cerebellar peduncle. The olives are a key distinguishing feature of the medulla.

The spinothalamic tract and the descending hypothalamic fibers course together in the lateral part of the medulla below the inferior cerebellar peduncle and near the spinal nucleus and tract of CN V.

Cranial Nerve Nuclei

Spinal nucleus of V

The spinal nucleus of the trigeminal nerve (CN V) is located in a position analogous to the dorsal horn of the spinal cord. The spinal tract of the trigeminal nerve lies just lateral to this nucleus and extends from the upper cervical cord (C2) to the point of entry of the fifth cranial nerve in the pons. Central processes from cells in the trigeminal ganglion conveying pain and temperature sensations from the face enter the brain stem in the rostral pons but descend in the spinal tract of CN V and synapse on cells in the spinal nucleus.

Solitary nucleus

The solitary nucleus receives the axons of all general and special visceral afferent fibers carried into the CNS by CN VII, IX, and X. These include taste, cardiorespiratory, and gastrointestinal sensations carried by these cranial nerves. Taste and visceral sensory neurons all have their cell bodies in ganglia associated with CN VII, IX, and X outside the CNS.

Nucleus ambiguus

The nucleus ambiguus is a column of large motoneurons situated dorsal to the inferior olive. Axons arising from cells in this nucleus course in the ninth and tenth cranial nerves. The component to the ninth nerve is insignificant. In the tenth nerve, these fibers supply muscles of the soft palate, larynx, pharynx, and upper esophagus. A unilateral lesion will produce ipsilateral paralysis of the soft palate causing the uvula to deviate away from the lesioned nerve and nasal regurgitation of liquids, weakness of laryngeal muscles causing hoarseness, and pharyngeal weakness resulting in difficulty in swallowing.

Dorsal motor nucleus of CN X

These visceral motoneurons of CN X are located lateral to the hypoglossal nucleus in the floor of the fourth ventricle. This is a major parasympathetic nucleus of the brain stem, and it supplies preganglionic fibers innervating terminal ganglia in the thorax and the foregut and midgut parts of the gastrointestinal tract.

Hypoglossal nucleus

The hypoglossal nucleus is situated near the midline just beneath the central canal and fourth ventricle. This nucleus sends axons into the hypoglossal nerve to innervate all of the tongue muscles except the palatoglossus.

The accessory nucleus

The accessory nucleus is found in the cervical spinal cord. The axons of the spinal accessory nerve arise from the accessory nucleus, pass through the foramen magnum to enter the cranial cavity, and join the fibers of the vagus to exit the cranial cavity through the jugular foramen. As a result, intramedullary lesions do not affect fibers of the spinal accessory nerve. The spinal accessory nerve supplies the sternocleidomastoid and trapezius muscles.

The rootlets of the glossopharyngeal (CN IX) and vagus (CN X) nerves exit between the olive and the fibers of the inferior cerebellar peduncle. The hypoglossal nerve (CN XII) exits more medially between the olive and the medullary pyramid.

PONS

The pons is located between the medulla (caudally) and the midbrain (rostrally). The cerebellum overlies the pons. It is connected to the brain stem by 3 pairs of cerebellar peduncles. The fourth ventricle is found between the dorsal surface of the pons and the cerebellum. The ventral surface of the pons is dominated by fibers, which form a large ventral enlargement that carries fibers from pontine nuclei to the cerebellum in the middle cerebellar peduncle. This ventral enlargement is the key distinguishing feature of the pons.

The corticospinal tracts are more diffuse in the pons than in the medulla and are embedded in the transversely coursing fibers that enter the cerebellum in the middle cerebellar peduncle.

The medial lemniscus is still situated near the midline but is now separated from the corticospinal tracts by the fibers forming the middle cerebellar peduncle. The medial lemniscus has changed from a dorsoventral orientation in the medulla to a more horizontal orientation in the pons.

The spinothalamic tract and the descending hypothalamic fibers continue to course together in the lateral pons.

The lateral lemniscus, an ascending auditory pathway, is lateral and just dorsal to the medial lemniscus. The lateral lemniscus carries the bulk of ascending auditory fibers from both cochlear nuclei to the inferior colliculus of the midbrain.

The medial longitudinal fasciculus (MLF) is located near the midline just beneath the fourth ventricle.

Cranial Nerve Nuclei

Abducens nucleus

The abducens nucleus is found near the midline in the floor of the fourth ventricle just lateral to the MLF.

Facial motor nucleus

The facial motor nucleus is located ventrolateral to the abducens nucleus. Fibers from the facial nucleus curve around the posterior side of the abducens nucleus (the curve forms the internal genu of the facial nerve), then pass ventrolaterally to exit the brain stem at the pontomedullary junction.

Superior olivary nucleus

The superior olivary nucleus lies immediately ventral to the nucleus of CN VII and receives auditory impulses from both ears by way of the cochlear nuclei. The cochlear nuclei are found at the pontomedullary junction just lateral to the inferior cerebellar peduncle.

Vestibular nuclei

The vestibular nuclei are located near the posterior surface of the pons lateral to the abducens nucleus, and extend into the medulla.

Cochlear nuclei

The dorsal and ventral cochlear nuclei are found at the pontomedullary junction. All of the fibers of the cochlear part of CN VIII terminate here.

Trigeminal nuclei

Motor Nucleus—Pons

The motor nucleus of CN V is located in the pons just medial to the main sensory nucleus of the trigeminal and adjacent to the point of exit or entry of the trigeminal nerve fibers. These motor fibers supply the muscles of mastication (masseter, temporalis, and medial and lateral pterygoid (Figure IV-5-3).

Main Sensory Nucleus—Pons

The main sensory nucleus is located just lateral to the motor nucleus.

The main sensory nucleus receives tactile and pressure sensations from the face, scalp, oral cavity, nasal cavity, and dura.

Spinal Trigeminal Nucleus—Spinal cord to pons

The spinal trigeminal nucleus is a caudal continuation of the main sensory nucleus, extending from the mid pons through the medulla to the cervical cord. Central processes from cells in the trigeminal ganglion conveying pain and temperature sensations from the face descend in the spinal tract of V and synapse on cells in the spinal nucleus.

Note

VPM relays touch, pain, temperature (CN V) and taste (CN VII, IX) sensations to cortex.

Mesencephalic Nucleus—Midbrain

The mesencephalic nucleus of CN V is located at the point of entry of the fifth nerve and extends into the midbrain. It receives proprioceptive input from joints, muscles of mastication, extraocular muscles, teeth, and the periodontium. Some of these fibers synapse monosynaptically on the motoneurons, forming the sensory limb of the jaw jerk reflex.

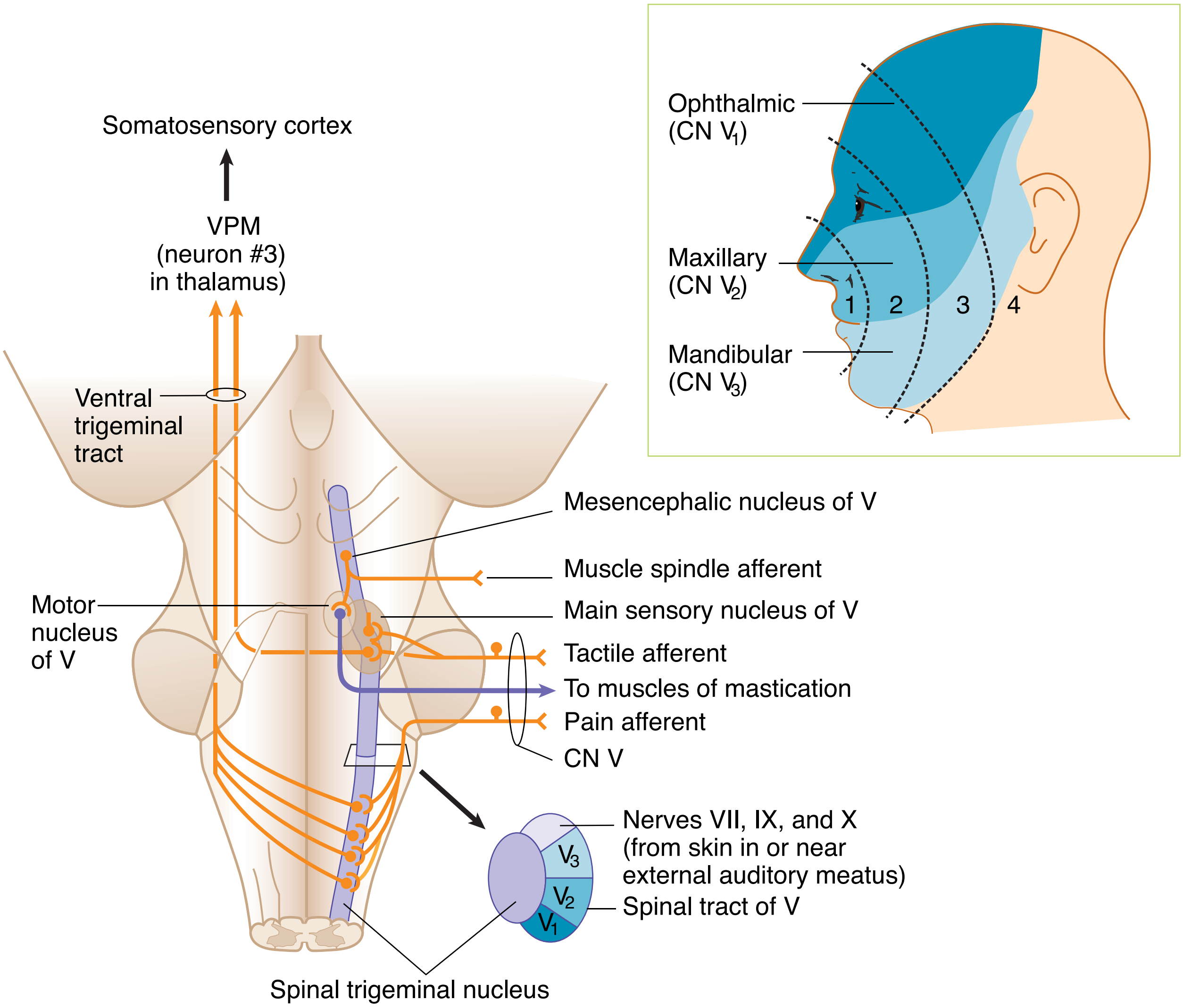


Figure III.5.5 Shaded areas indicate regions of face and scalp innervated by branches of the 3 divisions of CN V.

Dotted lines indicate concentric numbered "onion-skin" regions emanating posteriorly from nose and mouth that have a rostral to caudal representation in the spinal nucleus of V in the brain stem.

Cranial Nerves V, VI, VII, and VIII

Four cranial nerves emerge from the pons. Cranial nerves VI, VII, and VIII emerge from the pontomedullary junction. The facial nerve is located medial to the vestibulocochlear nerve. The abducens nerve (CN VI) emerges near the midline lateral to the corticospinal tract. The trigeminal nerve (CN V) emerges from the middle of the pons.

MIDBRAIN

The midbrain (mesencephalon) is located between the pons and diencephalon. The cerebral aqueduct, a narrow channel that connects the third and fourth ventricles, passes through the midbrain. The inferior colliculi and superior colliculi are found on the dorsal aspect of the midbrain above the cerebral aqueduct. The inferior colliculus processes auditory information received bilaterally from the cochlear nuclei by axon fibers of the lateral lemniscus. The superior colliculi help direct movements of both eyes in gaze. The pretectal region is located just beneath the superior colliculi and in front of the oculomotor complex. This area contains interneurons involved in the pupillary light reflex. The massive cerebral peduncles extend ventrally from the midbrain. The cerebral peduncles contain corticospinal and corticobulbar fibers. The interpeduncular fossa is the space between the cerebral peduncles.

The substantia nigra is the largest nucleus of the midbrain. It appears black to dark brown in the freshly cut brain because nigral cells contain melanin pigments. Neurons in the substantia nigra utilize Dopamine and GABA as neurotransmitters.

The medial lemniscus and spinothalamic tract and descending hypothalamic fibers course together ventrolateral to the periaqueductal gray.

The MLF continues to be located near the midline, just beneath the cerebral aqueduct.

The mesencephalic nuclei of the trigeminal nerve are located on either side of the central gray.

Cranial Nerve Nuclei

The trochlear nucleus is located just beneath the periaqueductal gray near the midline between the superior and inferior colliculi. The oculomotor nucleus and the nucleus of Edinger-Westphal are found just beneath the periaqueductal gray near the midline at the level of the superior colliculi.

Two cranial nerves emerge from the midbrain: the oculomotor (CN III) and the trochlear (CN IV) nerves.

The **oculomotor nerve** arises from the oculomotor nucleus and exits ventrally from the midbrain in the interpeduncular fossa. CN III also contains preganglionic parasympathetic axons that arise from the nucleus of Edinger-Westphal, which lies adjacent to the oculomotor nucleus.

Axons of the **trochlear nerve** decussate in the superior medullary velum and exit the brain stem near the posterior midline just inferior to the inferior colliculi.

Corticobulbar (Corticonuclear) Innervation of Cranial Nerve Nuclei

Corticobulbar fibers serve as the source of upper motoneuron innervation of lower motoneurons in cranial nerve nuclei. Corticobulbar fibers arise in the motor cortex and influence lower motoneurons in all brain stem nuclei that innervate skeletal muscles. This includes:

- Muscles of mastication (CN V)
- Muscles of facial expression (CN VII) – (partially bilateral)
- Palate, pharynx, and larynx (CN X)
- Tongue (CN XII)
- Sternocleidomastoid and trapezius muscles (CN XI)

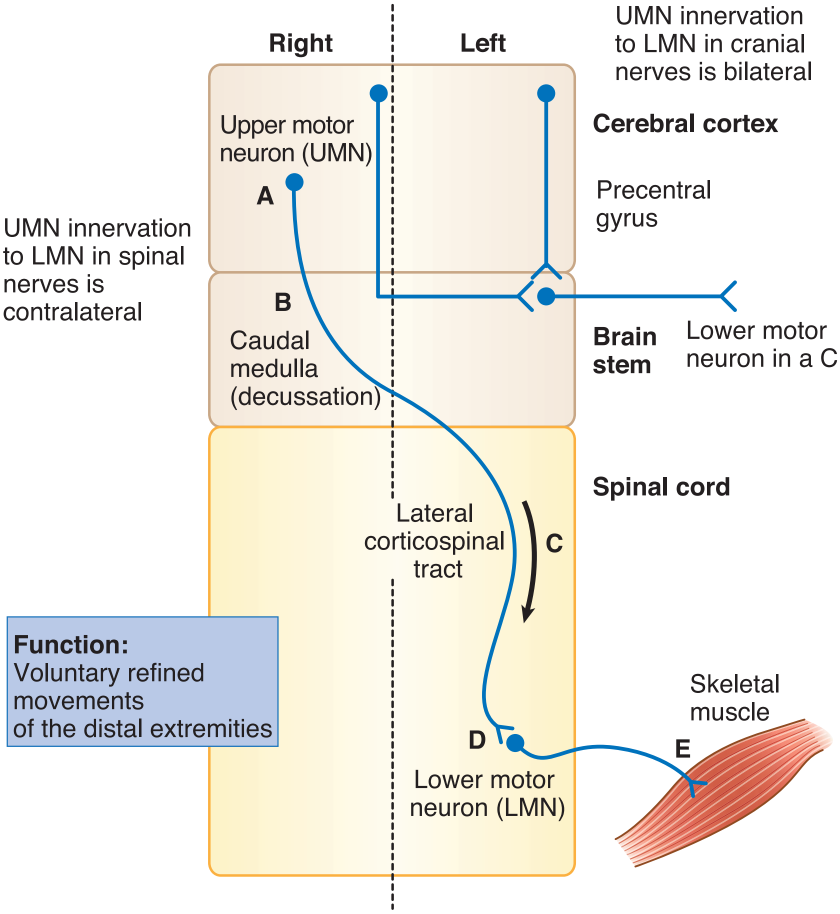


Figure III-5-6. Upper Motor Neuron Innervation of Spinal Nerves and Cranial Nerves

The corticobulbar innervation of cranial nerve lower motoneurons is predominantly bilateral, in that each lower motoneuron in a cranial nerve nucleus receives input from corticobulbar axons arising from both the right and the left cerebral cortex. T he major exception is that only some of the LMNs of the facial nerve (CN VII) receive a contralateral innervation.

Clinical Correlate

Facial Paralysis

The upper motoneuron innervation of lower motoneurons in the facial motor nucleus is different and clinically significant. Like most cranial nerve lower motoneurons, the corticobulbar innervation of facial motoneurons to muscles of the upper face (which wrinkle the forehead and shut the eyes) is bilateral. The corticobulbar innervation of facial motoneurons to muscles of the mouth, however, is contralateral only. Clinically, this means that one can differentiate between a lesion of the seventh nerve and a lesion of the corticobulbar fibers to the facial motor nucleus.

A facial nerve lesion (as in **Bell Palsy**) will result in a complete ipsilateral paralysis of muscles of facial expression, including an inability to wrinkle the forehead or shut the eyes and a drooping of the corner of the mouth. A corticobulbar lesion will result in only a drooping of the corner of the mouth on the contralateral side of the face and no other facial motor deficits. Generally, no other cranial deficits will be seen with corticobulbar lesions because virtually every other cranial nerve nucleus is bilaterally innervated. In some individuals, the hypoglossal nucleus may receive mainly contralateral corticobulbar innervation. If these corticobulbar fibers are lesioned, the tongue muscles undergo transient weakness without atrophy or fasciculations and may deviate away from the injured corticobulbar fibers. If, for example, the lesion is in corticobulbar fibers on the left, there is transient weakness of the right tongue muscles, causing a deviation of the tongue toward the right side upon protrusion.

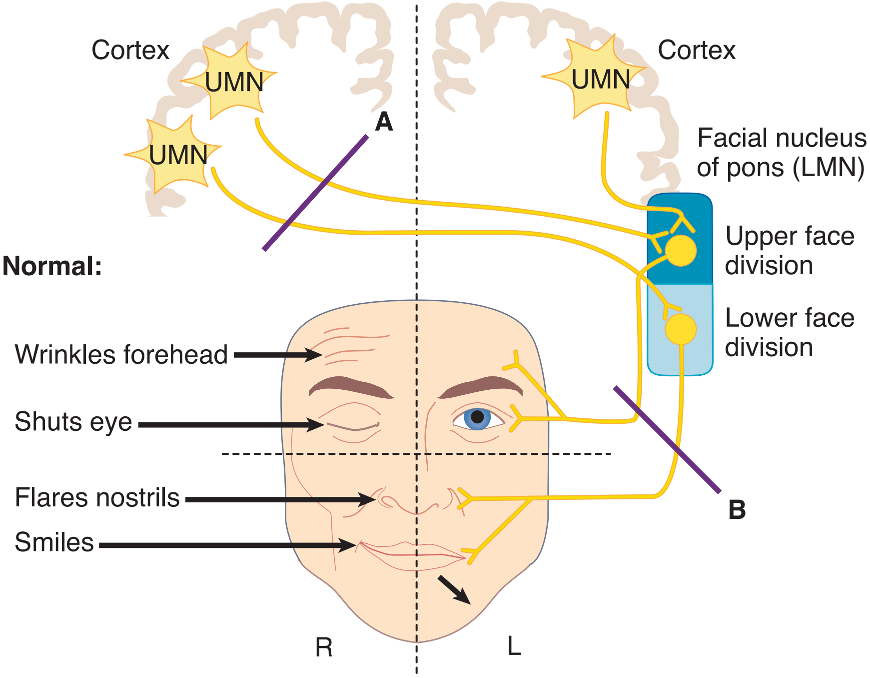


Figure III-5-7. Corticobulbar Innervation of the Facial Motor Nucleus

Clinical Correlate

Lesion A: left lower face weakness

Lesion B: complete left face weakness

Abbreviations

UMN = upper motoneuron

LMN = lower motoneuron

EAR, AUDITORY, AND VESTIBULAR SYSTEMS

Each ear consists of 3 components: 2 air-filled spaces, the external ear and the middle ear; and the fluid-filled spaces of the inner ear.

The external ear includes the pinna and the external auditory meatus, which extends to the tympanic membrane. Sound waves travel through the external auditory canal and cause the tympanic membrane (eardrum) to vibrate. Movement of the eardrum causes vibrations of the ossicles in the middle ear (i.e., the malleus, incus, and stapes). Vibrations of the ossicles are transferred through the oval window and into the inner ear.

The middle ear lies in the temporal bone, where the chain of 3 ossicles connects the tympanic membrane to the oval window. These auditory ossicles amplify the vibrations received by the tympanic membrane and transmit them to the fluid of the inner ear with minimal energy loss. The malleus is inserted in the tympanic membrane, and the stapes is inserted into the membrane of the oval window. Two small skeletal muscles, the tensor tympani and the stapedius, contract to prevent damage to the inner ear when the ear is exposed to loud sounds. The middle-ear cavity communicates with the nasopharynx via the eustachian tube, which allows air pressure to be equalized on both sides of the tympanic membrane.

The inner ear consists of a labyrinth (osseous and membranous) of interconnected sacs (utricle and saccule) and channels (semicircular ducts and the cochlear duct) that contain patches of receptor or hair cells that respond to airborne vibrations or movements of the head. Both the cochlear duct and the sacs and channels of the vestibular labyrinth are filled with endolymph, which bathes the hairs of the hair cells. Endolymph is unique because it has the inorganic ionic composition of an intracellular fluid but it lies in an extracellular space. The intracellular ionic composition of endolymph is important for the function of hair cells. Perilymph, ionically like a typical extracellular fluid, lies outside the endolymph-filled labyrinth.

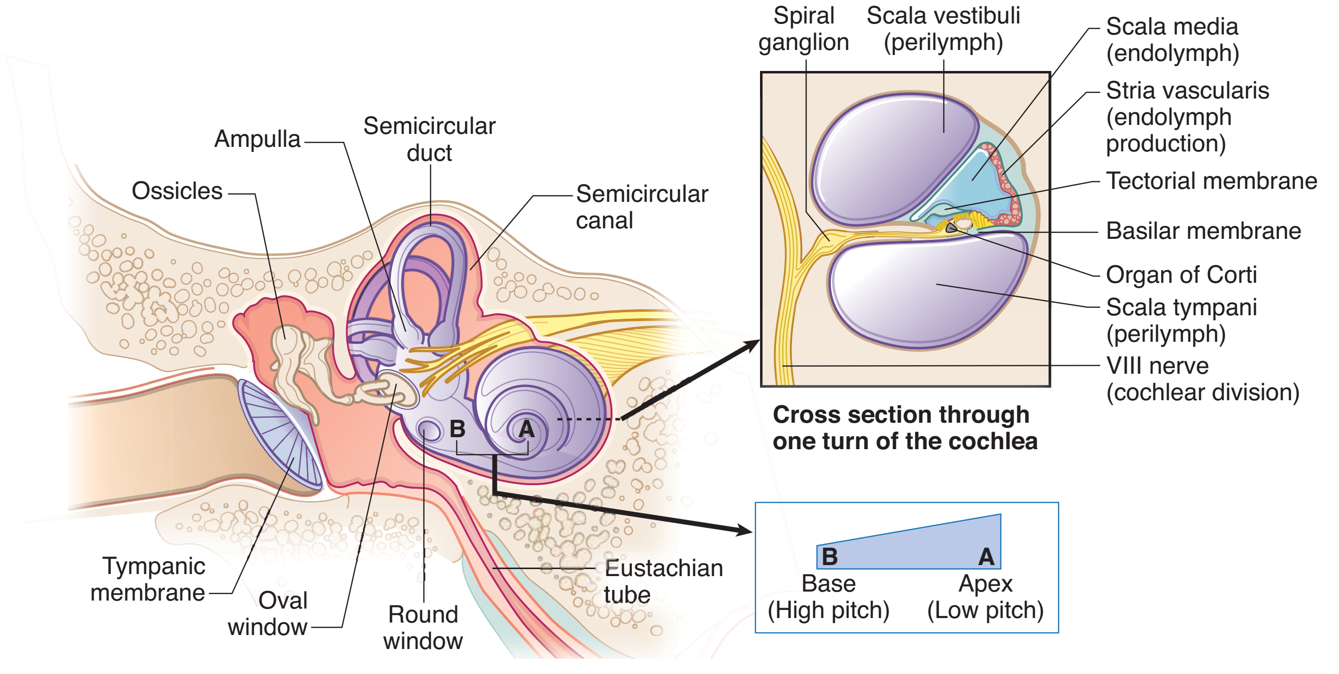


Figure III-5-8. Structures of the Inner Ear

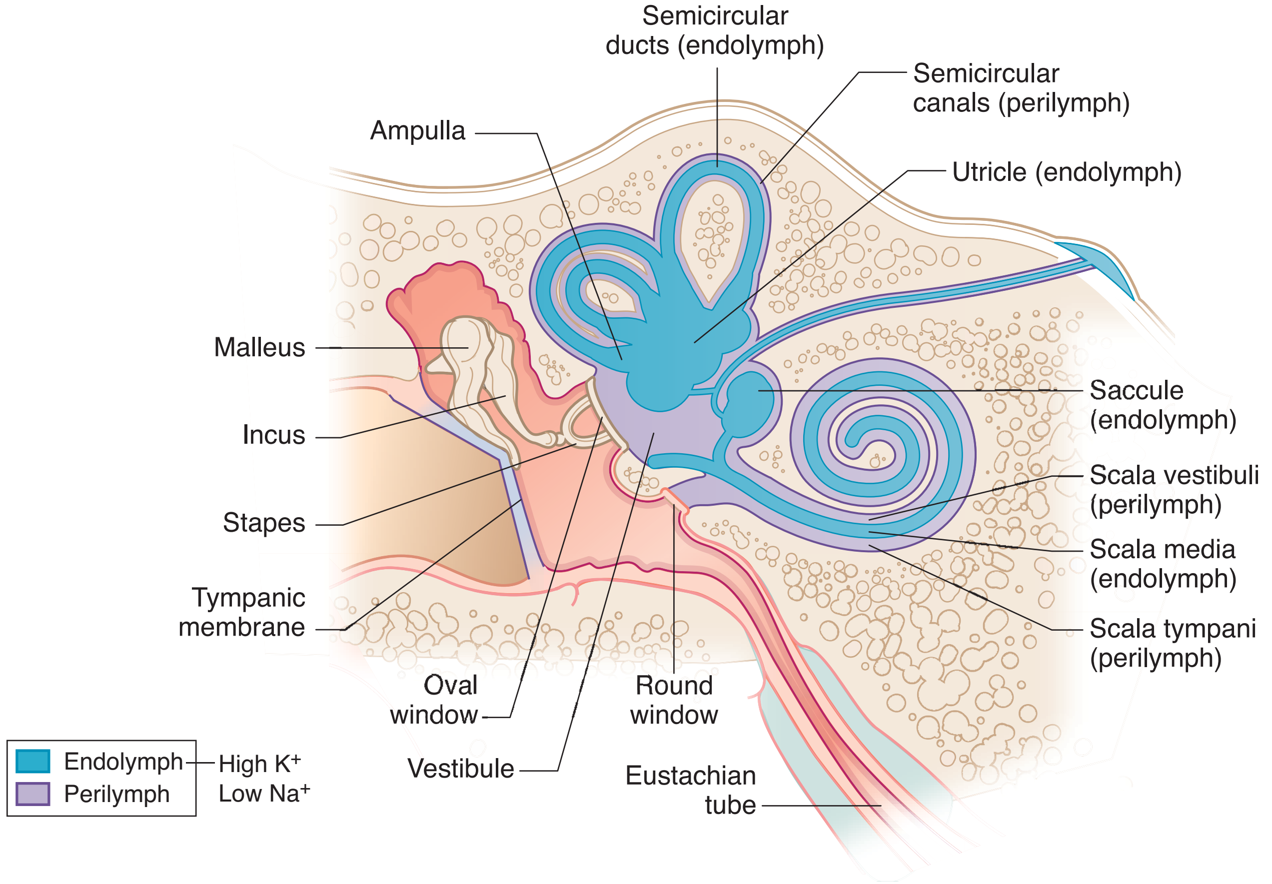


Figure III-5-9. Distribution of Endolymph and Perilymph in Inner Ear

Auditory System

The **cochlear duct** is the auditory receptor of the inner ear. It contains hair cells, which respond to airborne vibrations transmitted by the ossicles to the oval window. The cochlear duct coils 2 and a quarter turns within the bony cochlea and contains hair cells situated on an elongated, highly flexible, basilar membrane. High-frequency sound waves cause maximum displacement of the basilar membrane and stimulation of hair cells at the base of the cochlea, whereas low-frequency sounds maximally stimulate hair cells at the apex of the cochlea.

The **spiral ganglion** contains cell bodies whose peripheral axons innervate auditory hair cells of the organ of Corti. The central axons from these bipolar cells form the cochlear part of the eighth cranial nerve. All of the axons in the cochlear part of the eighth nerve enter the pontomedullary junction and synapse in the ventral and dorsal cochlear nuclei. Axons of cells in the ventral cochlear nuclei bilaterally innervate the superior olivary nuclei in the pons. The superior olivary nuclei are the first auditory nuclei to receive binaural input and use the binaural input to localize sound sources. The lateral lemniscus carries auditory input from the cochlear nuclei and the superior olivary nuclei to the inferior colliculus in the midbrain. Each lateral lemniscus carries information derived from both ears; however, input from the contralateral ear predominates.

Clinical Correlate

Middle-ear diseases (otitis media, otosclerosis) result in a conductive hearing loss because of a reduction in amplification provided by the ossicles.

Lesions of the facial nerve in the brain stem or temporal bone (Bell palsy) may result in hyperacusis, an increased sensitivity to loud sounds.

Clinical Correlate

Presbycusis results from a loss of hair cells at the base of the cochlea.

Clinical Correlate

Sensorineural hearing loss: air conduction > bone conduction

Conductive hearing loss: bone conduction > air conduction

Clinical Correlate

Lesions Causing Hearing Loss

Lesions of the cochlear part of the eighth nerve or cochlear nuclei inside the brain stem at the pontomedullary junction result in a profound unilateral sensorineural hearing loss (A). All other lesions to auditory structures in the brain stem, thalamus, or cortex result in a bilateral suppression of hearing and a decreased ability to localize a sound source (B). If a patient presents with a significant hearing loss in one ear, the lesion is most likely in the middle ear, inner ear, eighth nerve, or cochlear nuclei, and not at higher levels of the auditory system.

The **inferior colliculus** sends auditory information to the medial geniculate body (MGB) of the thalamus. From the MGB, the auditory radiation projects to the primary auditory cortex located on the posterior portion of the transverse temporal gyrus (Heschl's gyrus; Brodmann areas 41 and 42). The adjacent auditory association area makes connections with other parts of the cortex, including Wernicke's area, the cortical area for the comprehension of language.

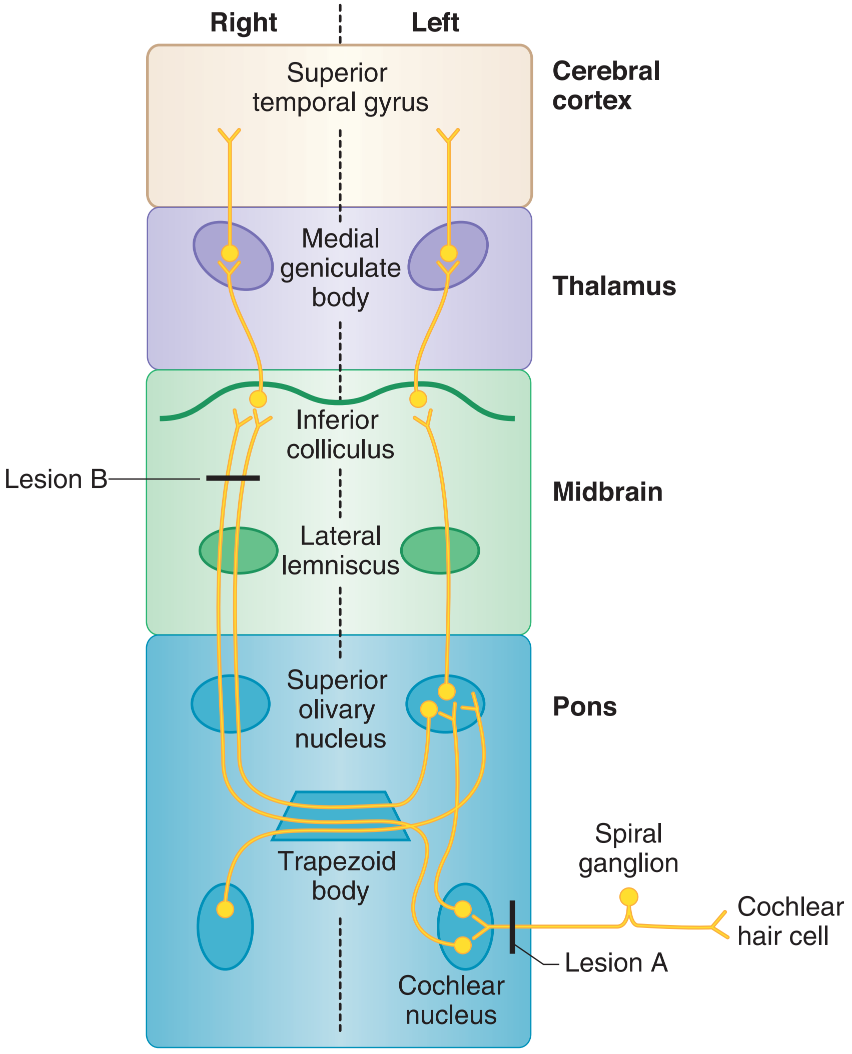


Figure III-5-10. Auditory System

Hearing Loss

Conductive: passage of sound waves through external or middle ear is interrupted. Causes: obstruction, otosclerosis, otitis media

Sensorineural: damage to cochlea, CN VIII, or central auditory connections

Auditory Tests

Weber test: place tuning fork on vertex of skull. If **unilateral conductive loss** → vibration is louder in affected ear; if **unilateral sensorineural loss** → vibration is louder in normal ear.

Rinne test: place tuning fork on mastoid process (bone conduction) until vibration is not heard, then place fork in front of ear (air conduction). If **unilateral conductive loss** → no air conduction after bone conduction is gone; if **unilateral sensorineural loss** → air conduction present after bone conduction is gone.

Vestibular System

Sensory receptors

The vestibular system contains 2 kinds of sensory receptors, one kind in the utricle and the saccule and the other in the semicircular ducts.

The utricle and the saccule are 2 large sacs, each containing a patch of hair cells in a macula. Each macula responds to linear acceleration and detects positional changes in the head relative to gravity. There are 3 semicircular ducts in the inner ear, each lying in a bony semicircular canal. Each semicircular duct contains an ampullary crest of hair cells that detect changes in angular acceleration resulting from circular movements of the head. The 3 semicircular ducts—anterior, posterior, and horizontal—are oriented such that they lie in the 3 planes of space. Circular movements of the head in any plane will depolarize hair cells in a semicircular duct in one labyrinth and hyperpolarize hair cells in the corresponding duct in the opposite labyrinth.

Vestibular nuclei

There are 4 vestibular nuclei located in the rostral medulla and caudal pons. The vestibular nuclei receive afferents from the vestibular nerve, which innervates receptors located in the semicircular ducts, utricle, and saccule. Primary vestibular fibers terminate in the vestibular nuclei and the flocculonodular lobe of the cerebellum.

Vestibular fibers

Secondary vestibular fibers, originating in the vestibular nuclei, join the MLF and supply the motor nuclei of CN III, IV, and VI. These fibers are involved in the production of conjugate eye movements. These compensatory eye movements represent the efferent limb of the vestibulo-ocular reflex, which enables the eye to remain focused on a stationary target during movement of the head or neck. Most of our understanding of the vestibulo-ocular reflex is based on horizontal head turning and a corresponding horizontal movement of the eyes in the direction opposite to that of head turning. For example, when the head turns horizontally to the right, both eyes will move to the left using the following vestibulo-ocular structures. Head turning to the right stimulates hairs cells in the right semicircular ducts. The right eighth nerve increases its firing rate to the right vestibular nuclei. These nuclei then send axons by way of the MLF to the right oculomotor nucleus and to the left abducens nucleus. The right oculomotor nerve to the right medial rectus adducts the right eye, and the left abducens nerve to the left lateral rectus abducts the left eye. The net effect of stimulating these nuclei is that both eyes will look to the left.

Note

Vestibular Functions:

- Equilibrium
- Posture
- VOR

Clinical Correlate

A lesion of the vestibular nuclei or nerve (in this example, on the left) produces a vestibular nystagmus with a slow deviation of the eyes toward the lesion and a fast correction back to the right.

Vestibular System (VIII)

Three semicircular ducts respond to **angular acceleration and deceleration** of the head. The **utricle** and **saccule** respond to **linear acceleration** and the pull of **gravity**. There are 4 **vestibular nuclei** in the medulla and pons, which receive information from CN VIII. Fibers from the vestibular nuclei join the MLF and supply the motor nuclei of CNs III, IV, and VI, thereby regulating conjugate eye movements. Vestibular nuclei also receive and send information to the **flocculonodular lobe** of the cerebellum.

Vestibulo-Ocular Reflex

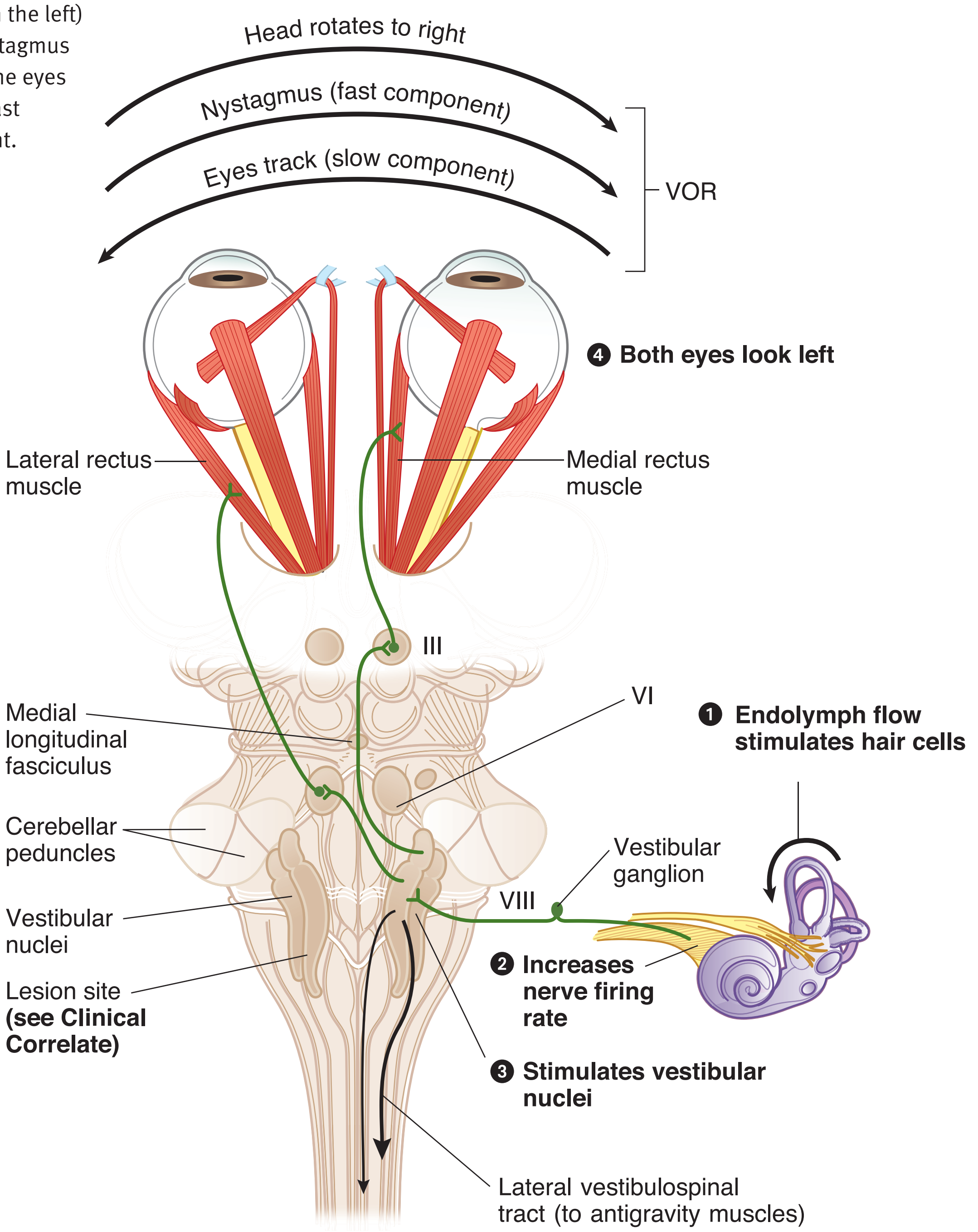


Figure III-5-11. Vestibulo-Ocular Reflex (VOR)

Caloric Test

This stimulates the horizontal semicircular ducts; can be used as a test of brain-stem function in unconscious patients.

Normal results:

- **Cold water** irrigation of ear → nystagmus to opposite side
- **Warm water** irrigation of ear → nystagmus to same side
- **COWS:** cold opposite, warm same

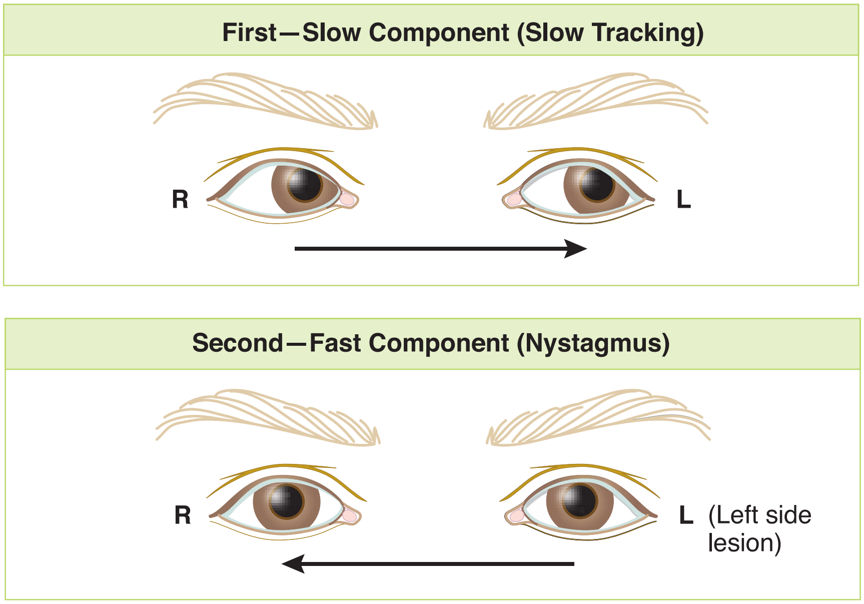


Figure III-5-12. Vestibular System

Clinical Correlate

Vestibular dysfunction may result from a peripheral or central lesion.

Vertigo may result from a lesion of the peripheral (end organ, nerve) or central (nuclear, brain-stem pathways) vestibular structures. Vertigo refers to the perception of rotation, which may involve either the subject or the external space. The vertigo is usually severe in peripheral disease and mild in brain-stem disease. Chronic vertigo (i.e., persisting longer than 2–3 weeks) strongly suggests a central lesion. Vertigo may also be caused by a variety of drugs, including anticonvulsants, aspirin, alcohol, and certain sedatives and antibiotics.

Ménière disease is characterized by abrupt, recurrent attacks of vertigo lasting minutes to hours accompanied by tinnitus or deafness and usually involving only one ear. Nausea and vomiting and a sensation of fullness or pressure in the ear also are common during the acute episode. The attacks are often severe, and the patient may be unable to stand. The disease usually occurs in middle age and results from distention of the fluid spaces in the cochlear and vestibular parts of the labyrinth.

Eye Movement Control Systems

For the eyes to move together **(conjugate gaze)**, the oculomotor nuclei and abducens nuclei are interconnected by the **medial longitudinal fasciculus (MLF)**.

Horizontal gaze is controlled by 2 gaze centers: **frontal eye field** (contralateral gaze), and **PPRF** (paramedian pontine reticular formation, ipsilateral gaze).

Nystagmus

Nystagmus refers to rhythmic oscillations of the eyes slowly to one side followed by a rapid reflex movement in the opposite direction. Nystagmus is defined by the direction of the rapid reflex movement or the fast phase. It is usually horizontal, although rotatory or vertical nystagmus may also occur.

Unilateral vestibular nerve or vestibular nucleus lesions may result in a vestibular nystagmus. In a pathologic vestibular nystagmus, the initial slow phase is the response to the pathology, and the fast phase is the correction attempt made by the cortex in response to the pathology. Consider this example: if the left vestibular nerve or nuclei are lesioned, because of the loss of balance between the 2 sides, the right vestibular nuclei are unopposed and act as if they have been stimulated, causing both eyes to look slowly to the left. This is the slow phase of a pathologic vestibular nystagmus. Because the head did not move, the cortex responds by moving both eyes quickly back to the right, the direction of the fast phase of the nystagmus.

Note

To remember the direction of the fast phase of vestibular nystagmus in a caloric test **toward the warm-water side** and **away from the cool-water side**, remember the mnemonic COWS:

- Cool
- Opposite
- Warm
- Same

The integrity of the vestibulo-ocular reflex can be an indicator of brain-stem integrity in comatose patients. To test this reflex, a vestibular nystagmus is induced by performing a **caloric test** in which an examiner introduces warm or cool water into an external auditory meatus. Warm water introduced into the external ear stimulates the horizontal semicircular duct and causes the eyes to move slowly in the opposite direction. Because the head did not turn, the eyes are moved quickly back by the cortex (if intact) toward the same ear where the warm water was introduced, producing a fast phase of nystagmus to the same side. Introduction of cool water into the external ear mimics a lesion; the horizontal duct activity is inhibited on the cool water side, and the opposite vestibular complex moves the eyes slowly toward the cool-water ear. The corrective or fast phase of the nystagmus moves the eyes quickly away from the ear where the cool water was introduced.

HORIZONTAL CONJUGATE GAZE

The eyeballs move together in conjugate gaze. The ocular muscles function to move and position both eyes as a unit so that an image falls on a corresponding spot on the retina of each eye. The slightest weakness in the movements of one eye causes diplopia, the presence of a double image, indicating that the image has been shifted to a different position on the retina of the affected side. Although gaze in all planes is possible, the muscles and cranial nerves involved in horizontal conjugate gaze, or abduction and adduction of both eyes together, are the most important eye movements.

Abduction of each eyeball is performed largely by the lateral rectus muscle, which is innervated by the abducens nerve (CN VI). Adduction of the eyeball is performed by the medial rectus muscle, which is innervated by the oculomotor nerve (CN III). Therefore, for both eyes to look to the right in horizontal gaze, the right abducens nerve and the right lateral rectus muscle must be active to abduct the right eye, and the left oculomotor nerve and the left medial rectus muscle must be active to adduct the left eye. The net effect is that both eyes will look to the right.

In the brain stem, the abducens nucleus (CN VI) and the oculomotor nucleus (CN III) are situated close to the midline just beneath the fourth ventricle or the cerebral aqueduct, in the pons and midbrain. These nuclei are interconnected by the fibers in the MLF. It is the fibers in the MLF that permit conjugate gaze, either when the target moves or when the head moves, through their interconnections to gaze centers and the vestibular system.

Control of Horizontal Gaze

Horizontal gaze is controlled by 2 interconnected gaze centers. One control center is in the frontal lobe, the frontal eye field (Brodmann area 8). This area acts as a center for **contralateral** horizontal gaze. In the pons is a second gaze center, known as the pontine gaze center or the PPRF, the paramedian pontine reticular formation. This is a center for **ipsilateral** horizontal gaze. When activated by neurons in the frontal eye field, the pontine gaze center neurons send axons to synapse with cell bodies in the abducens nucleus, which is actually contained within the pontine gaze center. The pontine gaze center also sends axons that cross immediately and course in the contralateral MLF to reach the contralateral oculomotor nucleus. The net effect of stimulation of the left frontal eye field, therefore, is activation of the pontine gaze center on the right and a saccadic horizontal eye movement of both eyes to the right. Horizontal gaze to the right results from activation of the right abducens nucleus and the left oculomotor nucleus by fibers in the MLF.

Lesions in the MLF result in an internuclear ophthalmoplegia in which there is an inability to adduct one eye on attempted gaze to the opposite side. For example, a lesion in the right MLF results in an inability to adduct the right eye on an attempted gaze to the left. The left eye abducts normally but exhibits a nystagmus. If the MLF is lesioned bilaterally (as might be the case in multiple sclerosis), neither eye adducts on attempted gaze (Figures III-5-13 and III-5-14), and the abducting eye exhibits a nystagmus.

Lesion sites are indicated by 1–4.

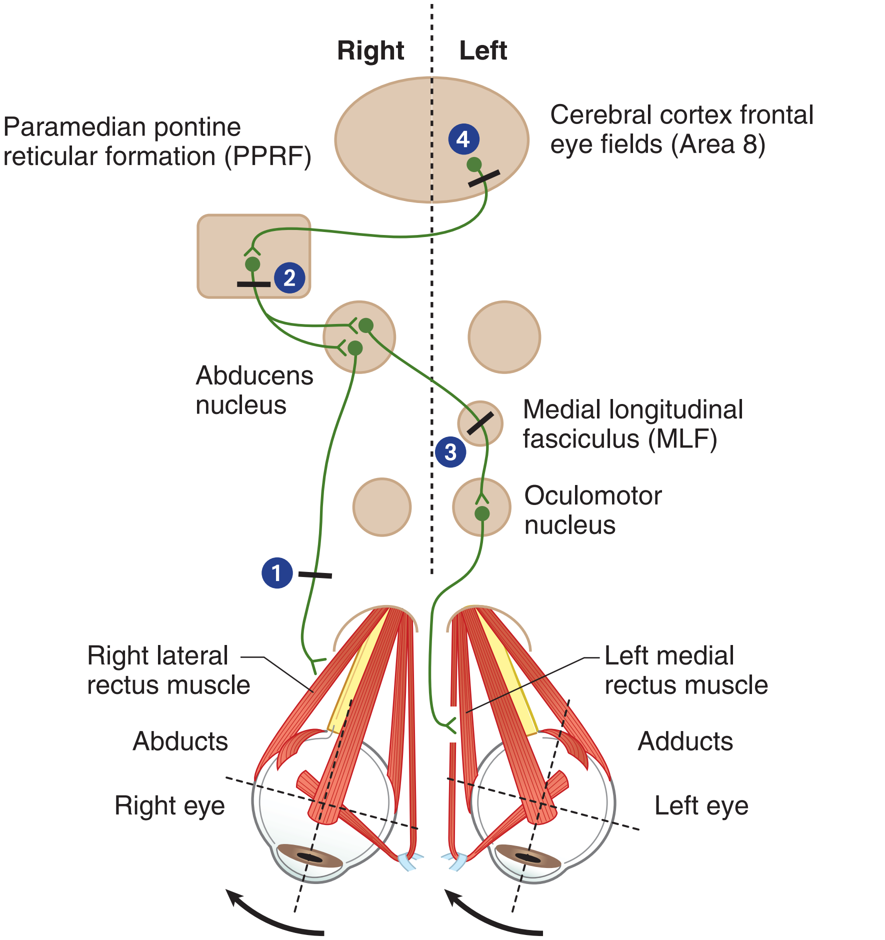


Figure III-5-13. Voluntary Horizontal Conjugate Gaze

Table III-5-2. Clinical Correlate

Lesion Examples	Symptoms
1. Right CN VI	Right eye cannot look right
2. Right PPRF	Neither eye can look right
3. Left MLF	**Internuclear ophthalmoplegia (INO)** Left eye cannot look right; convergence is intact (this is how to distinguish an INO from an oculomotor lesion); right eye has nystagmus; seen in multiple sclerosis
4. Left frontal eye field	Neither eye can look right; but slow drift to left

Abbreviations: MLF, medial longitudinal fasciculus; PPRF, paramedian pontine reticular formation

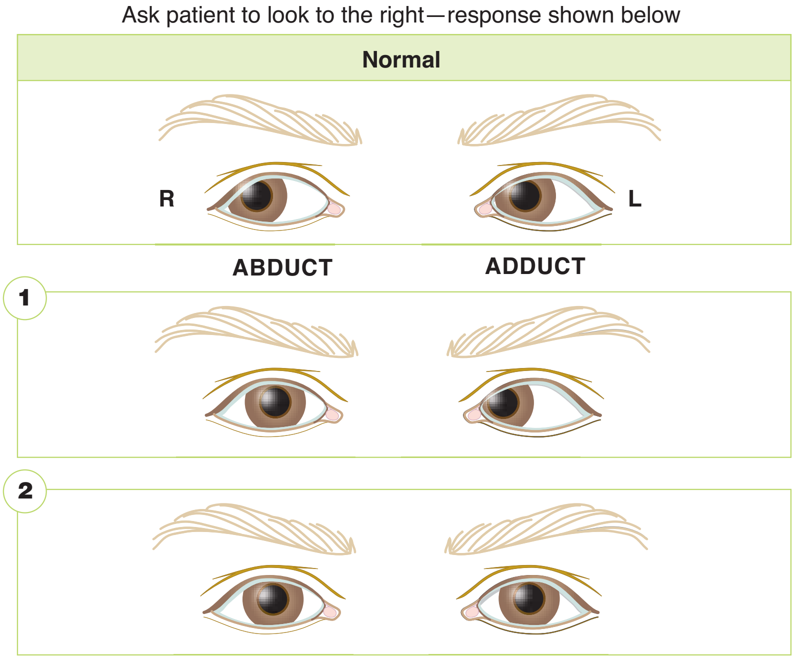


Figure III-5-14. Normal and Abnormal Horizontal Gaze

Clinical Correlate

The abducens nucleus is coexistent with the PPRF, the center for ipsilateral horizontal gaze. Lesions result in an inability to look to the lesion side, and may include a complete ipsilateral facial paralysis because the VIIth nerve fibers loop over the CN VI nucleus.

Table III-5-3. Normal/Abnormal Responses to Horizontal Conjugate Gaze: Part 1

Lesion Location	Symptoms (Results)
Right Abducens nerve, #1	Right eye cannot look right (abduct)
Right Abducens nucleus, #2	Neither eye can look right (lateral gaze paralysis)—may be slow drift left and complete **right facial paralysis**

Ask patient to look to the right—response shown below

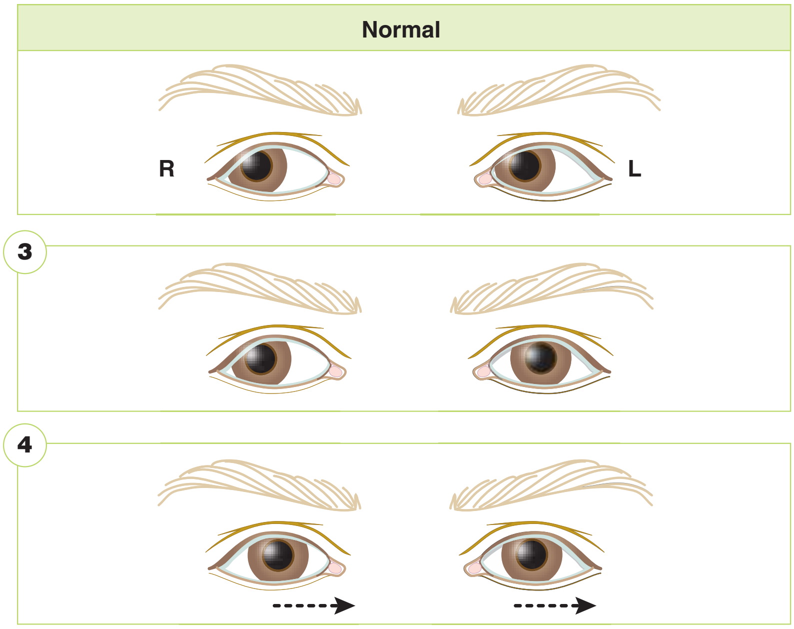


Figure III-5-15. Normal and Abnormal Horizontal Gaze

Table III-5-4. Normal/Abnormal Responses to Horizontal Gaze: Part 2

Lesion Location	Symptoms (Results)
Left MLF, #3 Internuclear ophthalmoplegia	Left eye cannot look right; convergence intact; right eye exhibits nystagmus
Left cerebral cortex, #4	Neither eye can look right: but slow drift to left; may be seen with right lower face weakness and right upper limb weakness

Abbreviation: MLF, medial longitudinal fasciculus

BLOOD SUPPLY TO THE BRAIN STEM

Vertebral Artery

This artery is a branch of the subclavian that ascends through the foramina of the transverse processes of the upper 6 cervical vertebrae. It enters the posterior fossa by passing through the foramen magnum. The vertebral arteries continue up the ventral surface of the medulla and, at the caudal border of the pons, join to form the basilar artery.

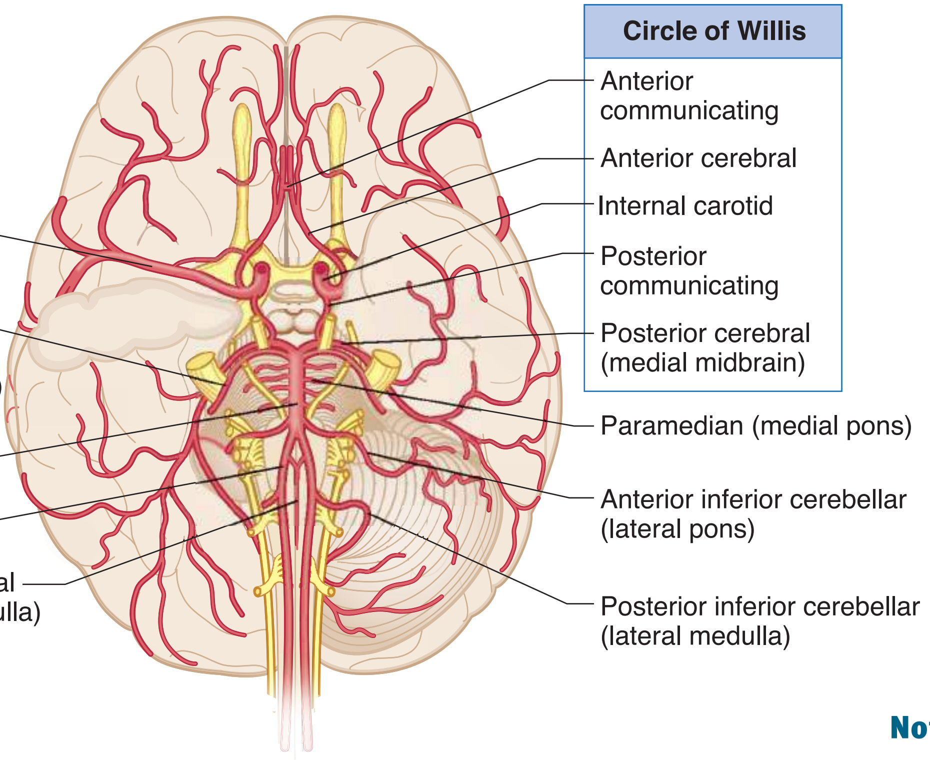


Figure III-5-16. Arterial Supply of the Brain

Branches of the vertebral artery include: the anterior spinal artery, which supplies the ventrolateral two-thirds of the cervical spinal cord and the ventromedial part of the medulla; and the posterior inferior cerebellar artery (PICA), which supplies the cerebellum and the dorsolateral part of the medulla.

Basilar Artery

The basilar artery is formed by the joining of the 2 vertebral arteries at the pontomedullary junction. It ascends along the ventral midline of the pons and terminates near the rostral border of the pons by dividing into the 2 posterior cerebral arteries. Branches include the anterior inferior cerebellar arteries (AICA) and the paramedian arteries.

Branches of the basilar artery include:the labyrinthine artery, which follows the course of the eighth cranial nerve and supplies the inner ear; the anterior inferior cerebellar artery, which supplies part of the pons and the anterior and inferior regions of the cerebellum; the superior cerebellar artery, which supplies part of the rostral pons and the superior region of the cerebellum; and pontine branches, which supply much of the pons via paramedian and circumferential vessels.

At the rostral end of the midbrain, the basilar artery divides into a pair of posterior cerebral arteries. Paramedian and circumferential branches of the posterior cerebral artery supply the midbrain.

Note

Brain stem:

Vertebral Artery:

1. ASA
2. PICA

Basilar:

1. AICA
2. Paramedian
3. Superior cerebellar
4. Posterior cerebral

BRAIN-STEM LESIONS

There are 2 keys to localizing brain-stem lesions. First, it is uncommon to injure parts of the brain stem without involving one or more cranial nerves. The cranial nerve signs will localize the lesion to the midbrain (CN III or IV), upper pons (CN V), lower pons (CN VI, VII, or VIII), or upper medulla (CN IX, X, or XII). Second, if the lesion is in the brain stem, the cranial nerve deficits will be seen with a lesion to one or more of the descending or ascending long tracts (corticospinal, medial lemniscus, spinothalamic, descending hypothalamic fibers). Lesions in the brain stem to any of the long tracts except for the descending hypothalamic fibers will result in a contralateral deficit. A unilateral lesion to the descending hypothalamic fibers that results in Horner syndrome is always seen ipsilateral to the side of the lesion.

Medial Medullary Syndrome

Medial medullary syndrome is most frequently the result of occlusion of the vertebral artery or the anterior spinal artery (Figure III-5-17). Medial medullary syndrome presents with a lesion of the hypoglossal nerve as the cranial nerve sign and lesions to both the medial lemniscus and the corticospinal tract. Corticospinal tract lesions produce contralateral spastic hemiparesis of both limbs.

Medial lemniscus lesions produce a contralateral deficit of proprioception and touch, pressure, and vibratory sensations in the limbs and body.

Lesions of the hypoglossal nerve in the medulla produce an ipsilateral paralysis of half the tongue with atrophy. Upon protrusion, the tongue deviates toward the side of the lesion.

Medulla

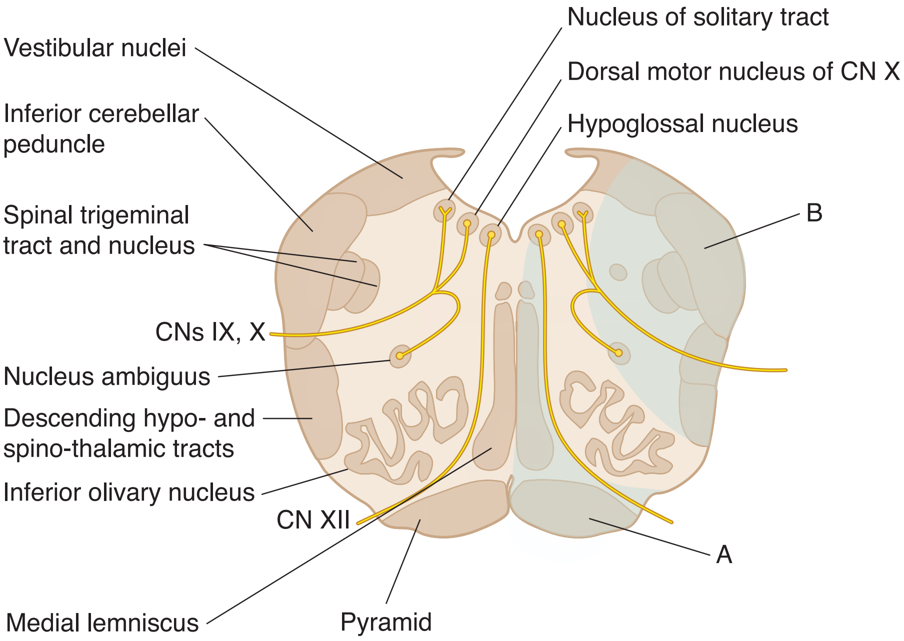


Figure III-5-17. Medulla Lesions

Medial Medullary Syndrome A

Anterior Spinal Artery

- **Pyramid:** contralateral spastic paresis
- **Medial lemniscus:** contralateral loss of tactile, vibration, conscious proprioception
- **XII nucleus/fibers:** ipsilateral flaccid paralysis of tongue with tongue deviation on protrusion to lesion side.

Lateral Medullary Syndrome B

PICA, Wallenberg Syndrome

- **Inferior cerebellar peduncle:** ipsilateral limb ataxia
- **Vestibular nuclei:** vertigo, nausea/vomiting, nystagmus (away from lesion)
- **Nucleus ambiguus (CN IX, X):** ipsilateral paralysis of larynx, pharynx, palate → dysarthria, dysphagia, loss of gag reflex
- **Spinal V:** ipsilateral pain/temperature loss (face)
- **Spinothalamic tract:** Contralateral pain/temperature loss (body)
- **Descending hypothalamics:** ipsilateral Horner syndrome

Lateral Medullary (Wallenberg) Syndrome

Lateral medullary syndrome results from occlusion of the PICA. The cranial nerves or nuclei involved in the lesion are the vestibular or the cochlear parts of CN VIII, the glossopharyngeal and the vagus nerves, and the spinal nucleus or tract of V. The long tracts involved are the spinothalamic tract and the descending hypothalamic fibers.

Spinothalamic tract lesions produce a pain and temperature sensation deficit in the contralateral limbs and body.

Lesions of descending hypothalamic fibers produce an ipsilateral Horner syndrome (i.e., miosis, ptosis, and anhidrosis).

Lesions of the vestibular nuclei and pathways may produce nystagmus, vertigo, nausea, and vomiting. If there is a vestibular nystagmus, the fast component will be away from the side of the lesion.

Lesions of the vagus nerves exiting the medulla may produce dysphagia (difficulty in swallowing) or hoarseness. The palate will droop on the affected side, and the uvula will deviate away from the side of the lesion.

Lesions of the glossopharyngeal nerve result in a diminished or absent gag reflex.

Lesions of the spinal tract and nucleus of the trigeminal nerve produce a loss of just pain and temperature sensations on the ipsilateral side of half the face. Touch sensations from the face and the corneal blink reflex will be intact. In lateral medullary syndrome, the pain and temperature losses are alternating; these sensations are lost from the face and scalp ipsilateral to the lesion but are lost from the contralateral limbs and trunk.

Taste sensations may be altered if the solitary nucleus is involved.

Medial Pontine Syndrome

Medial pontine syndrome results from occlusion of paramedian branches of the basilar artery. At a minimum, this lesion affects the exiting fibers of the abducens nerve and the corticospinal tract. The medial lemniscus may be affected if the lesion is deeper into the pons, and the facial nerve may be affected if the lesion extends laterally.

The long tract signs will be the same as in medial medullary syndrome, involving the corticospinal and medial lemniscus, but the abducens nerve and the facial nerve lesions localize the lesion to the caudal pons.

Corticospinal tract lesions produce contralateral spastic hemiparesis of both limbs.

Medial lemniscus lesions produce a contralateral deficit of proprioception and touch, pressure, and vibratory sensations in the limbs and body.

Lesions of the abducens nerve exiting the caudal pons produce an internal strabismus of the ipsilateral eye (from paralysis of the lateral rectus). This results in diplopia on attempted lateral gaze to the affected side.

Lesions of the facial nerve exiting the caudal pons produce complete weakness of the muscles of facial expression on the side of the lesion.

Lesions of the facial nerve may also include an alteration of taste from the anterior two-thirds of the tongue, loss of lacrimation (eye dry and red), and loss of the motor limb of the corneal blink reflex.

If a lesion extends dorsally to include the abducens nucleus (which includes the horizontal gaze center in the PPRF), there may be a lateral gaze paralysis in which both eyes are forcefully directed to the side contralateral to the lesion.

Lateral Pontine Syndrome

Lesions of the dorsolateral pons usually result from occlusion of the anterior inferior cerebellar artery (caudal pons) or superior cerebellar artery (rostral pons). The long tracts involved will be the same as in lateral medullary syndrome, the spinothalamic tract and the descending hypothalamic fibers. The cranial nerves involved will be the facial and vestibulocochlear in the caudal pons, the trigeminal nerve in the rostral pons, and the spinal nucleus and tract of V in both lesions.

Spinothalamic tract lesions produce a pain and temperature sensation deficit in the contralateral limbs and body.

Lesions of descending hypothalamic fibers produce an ipsilateral Horner syndrome (i.e., miosis, ptosis, and anhidrosis).

Lesions of the vestibular nuclei and pathways (caudal pons) produce nystagmus, vertigo, nausea, and vomiting. Again, the fast phase of the nystagmus will be away from the side of the lesion. Lesions of the cochlear nucleus or auditory nerve produce an ipsilateral sensorineural hearing loss.

Lesions of the spinal tract and nucleus of the trigeminal nerve result only in a loss of pain and temperature sensations on the ipsilateral side of half the face.

Lesions of the facial nerve and associated structures produce ipsilateral facial paralysis, loss of taste from the anterior two-thirds of the tongue, loss of lacrimation and salivation, and loss of the corneal reflex.

Lesions of the trigeminal nerve (rostral pons) result in complete anesthesia of the face on the side of the lesion, weakness of muscles of mastication, and deviation of the jaw toward the lesioned side.

Pons

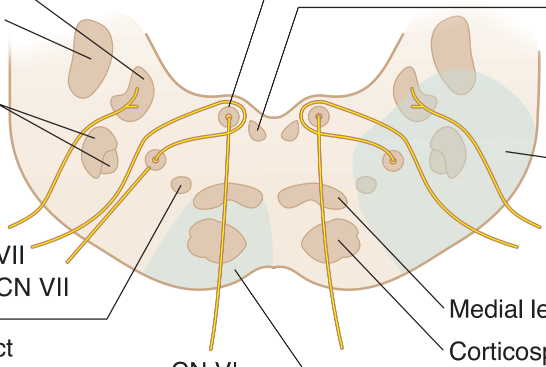


Figure III-5-18. Pons

Medial Pontine Syndrome (A)

Paramedian Branches of Basilar Artery

- **Corticospinal tract:** contralateral spastic hemiparesis
- **Medial lemniscus:** contralateral loss of tactile/position/vibration sensation
- **Fibers of VI:** medial strabismus

Lateral Pontine Syndrome (B)

AICA

- **Middle cerebellar peduncle:** ipsilateral ataxia
- **Vestibular nuclei:** vertigo, nausea and vomiting, nystagmus
- **Facial nucleus and fibers:** ipsilateral facial paralysis; ipsilateral loss of taste (anterior two-thirds of tongue), lacrimation, salivation, and corneal reflex; hyperacusis
- **Spinal trigeminal nucleus/tract:** ipsilateral pain/temperature loss (face)
- **Spinothalamic tract:** contralateral pain/temperature loss (body)
- **Cochlear nucleus/VIII fibers:** ipsilateral hearing loss
- **Descending hypothalamics:** ipsilateral Horner syndrome

Pontocerebellar Angle Syndrome

Pontocerebellar angle syndrome is usually caused by an acoustic neuroma (schwannoma) of CN VIII. This is a slow-growing tumor, which originates from Schwann cells in the vestibular nerve (or less commonly the auditory nerve). As the tumor grows, it exerts pressure on the lateral part of the caudal pons where CN VII emerges and may expand anteriorly to compress the fifth nerve. The cranial nerve deficits seen together localize the lesion to the brain stem, but the absence of long tract signs indicates that the lesion must be outside of the brain stem.

Medial Midbrain (Weber) Syndrome

Medial midbrain (Weber) syndrome results from occlusion of branches of the posterior cerebral artery. Exiting fibers of CN III are affected, along with corticobulbar and corticospinal fibers in the medial aspect of the cerebral peduncle. Third-nerve lesions result in a ptosis, mydriasis (dilated pupil), and an external strabismus. As with any brain-stem lesion affecting CN III, accommodation and convergence will also be affected. Corticospinal tract lesions produce contralateral spastic hemiparesis of both limbs. The involvement of the cortico-bulbar fibers results in a contralateral lower face weakness seen as a drooping of the corner of the mouth. The patient will be able to shut the eye (blink reflex is intact) and wrinkle the forehead.

Midbrain

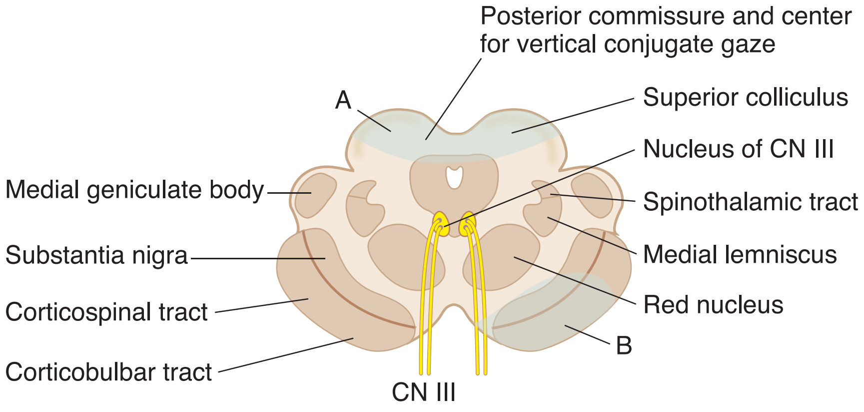


Figure III-5-19. Midbrain

Dorsal Midbrain (Parinaud) Syndrome (A)

Tumor in Pineal Region

- **Superior colliculus/pretectal area:** paralysis of upward gaze, various pupillary abnormalities
- **Cerebral aqueduct:** noncommunicating hydrocephalus

Medial Midbrain (Weber) Syndrome (B)

Branches of PCA

- **Fibers of III:** ipsilateral oculomotor palsy (lateral strabismus, dilated pupil, ptosis)
- **Corticospinal tract:** contralateral spastic hemiparesis
- **Corticobulbar tract:** contralateral hemiparesis of lower face

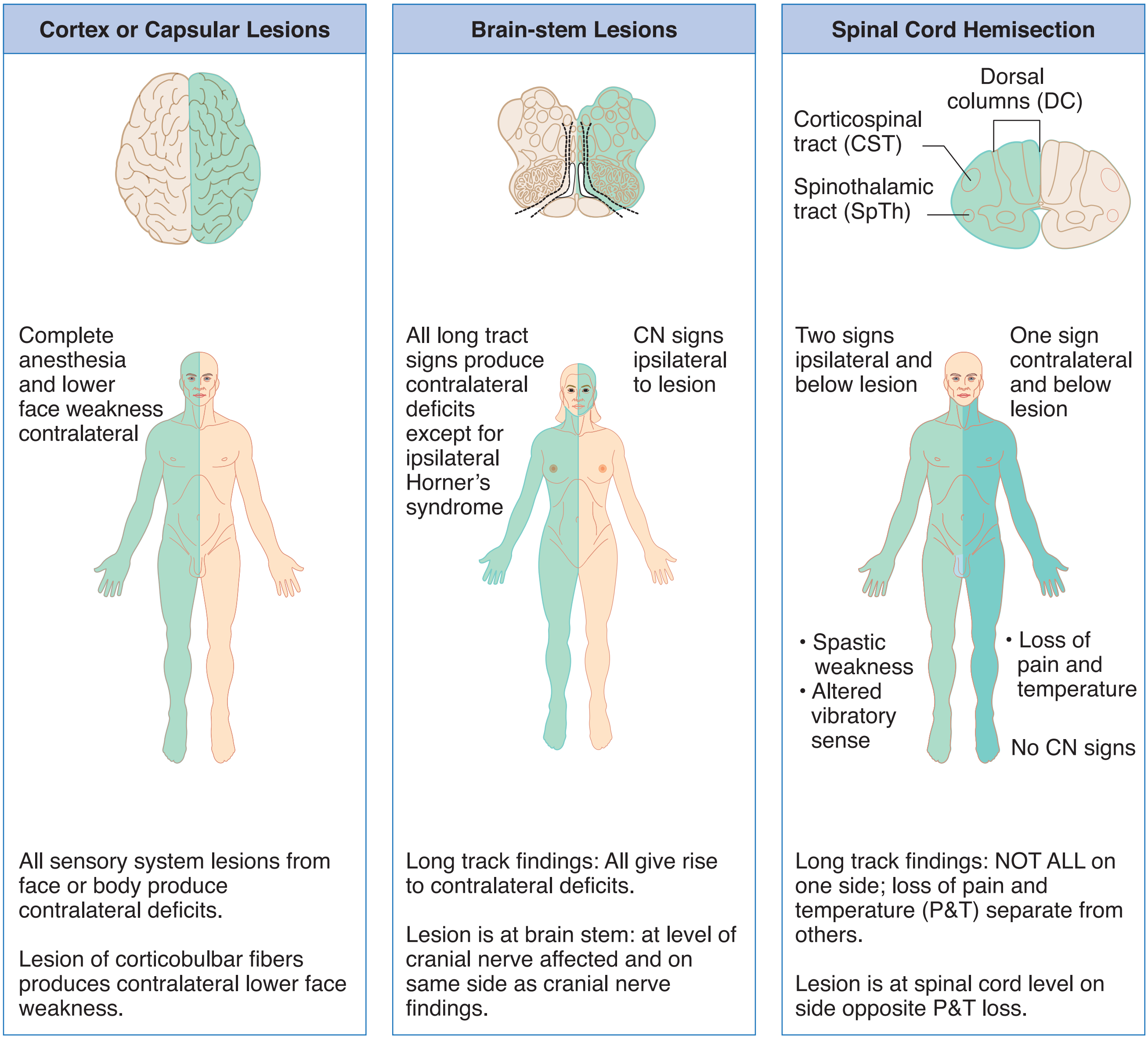


Figure III-5-20. Strategy for the Study of Lesions

Clinical Correlate

Neurons in both the raphe and locus caeruleus degenerate in Alzheimer disease.

Parinaud Syndrome

Parinaud syndrome usually occurs as a result of a pineal tumor compressing the superior colliculi. The most common sign is paralysis of upward or vertical gaze, combined with bilateral pupillary abnormalities (e.g., slightly dilated pupils, which may show an impaired light or accommodation reaction) and signs of elevated intracranial pressure. Compression of the cerebral aqueduct can result in noncommunicating hydrocephalus.

RETICULAR FORMATION

The reticular formation is located in the brain stem and functions to coordinate and integrate the actions of different parts of the CNS. It plays an important role in the regulation of muscle and reflex activity and control of respiration, cardiovascular responses, behavioral arousal, and sleep.

Reticular Nuclei

The **raphe nuclei** are a narrow column of cells in the midline of the brain stem, extending from the medulla to the midbrain. Cells in some of the raphe nuclei (e.g., the dorsal raphe nucleus) synthesize serotonin (5-hydroxytryptamine [5-HT]) from l-tryptophan and project to vast areas of the CNS. They play a role in mood, aggression, and the induction of non–rapid eye movement (non-REM) sleep.

Cells in the **locus caeruleus** synthesize norepinephrine and send projections to most brain areas involved in the control of cortical activation (arousal). Decreased levels of norepinephrine are evident in REM (paradoxic) sleep.

The **periaqueductal (central) gray** is a collection of nuclei surrounding the cerebral aqueduct in the midbrain. Opioid receptors are present on many periaqueductal gray cells, the projections from which descend to modulate pain at the level of the dorsal horn of the spinal cord.

The Cerebellum 6

Learning Objectives

- ❏ Use knowledge of general features
- ❏ Use knowledge of cerebellar cytoarchitecture
- ❏ Solve problems concerning circuitry

GENERAL FEATURES

The cerebellum, located dorsal to the pons and the medulla, is derived from the metencephalon. The fourth ventricle is found between the cerebellum and the dorsal aspect of the pons. The cerebellum functions in the planning and fine-tuning of skeletal muscle contractions; it performs these tasks by comparing an intended with an actual performance.

The cerebellum consists of a midline vermis and 2 lateral cerebellar hemispheres. The cerebellar cortex consists of multiple parallel folds (or folia) and contains several maps of the skeletal muscles in the body. The topographic arrangement of these maps indicates that the vermis controls the axial and proximal musculature of the limbs, the intermediate part of the hemisphere controls distal musculature, and the lateral part of the hemisphere is involved in motor planning.

The flocculonodular lobe is involved in control of balance and eye movements.

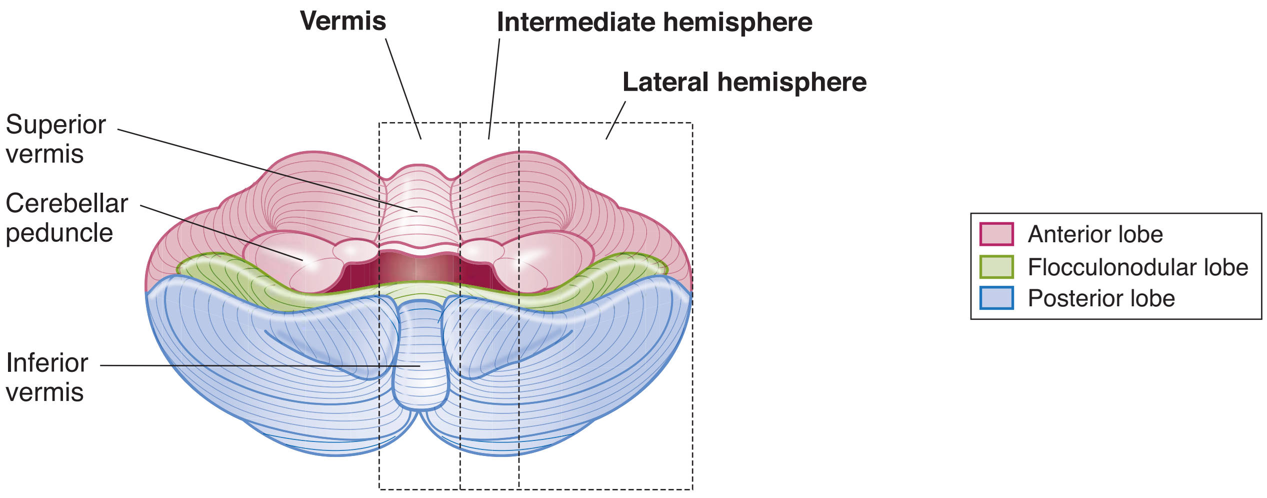


Figure III-6-1. Cerebellum

Table III-6-1. Cerebellum

Region	Function	Principle Input
Vermis and intermediate zones	Ongoing motor execution	Spinal cord
Hemisphere (lateral)	Planning/coordination	Cerebral cortex and inferior olivary nucleus
Flocculonodular lobe	Balance and eye movements	Vestibular nuclei (VIII)

Major input to the cerebellum travels in the inferior cerebellar peduncle (ICP) (restiform body) and middle cerebellar peduncle (MCP). Major outflow from the cerebellum travels in the superior cerebellar peduncle (SCP).

CEREBELLAR CYTOARCHITECTURE

All afferent and efferent projections of the cerebellum traverse the ICP, MCP, or SCP. Most afferent input enters the cerebellum in the ICP and MCP; most efferent outflow leaves in the SCP.

Table III-6-2. Major Afferents to the Cerebellum

Name	Tract	Enter Cerebellum Via	Target and Function
Mossy fibers	Vestibulocerebellar Spinocerebellar (Cortico) pontocerebellar	ICP ICP and SCP MCP (decussate)	Excitatory terminals on granule cells (glutamate)
Climbing fibers	Olivocerebellar	ICP (decussate)	Excitatory terminals on Purkinje cells

Abbreviations: ICP, inferior cerebellar peduncle; MCP, middle cerebellar peduncle; SCP, superior cerebellar peduncle

Internally, the cerebellum consists of an outer cortex and an internal white matter (medullary substance).

The 3 cell layers of the cortex are the molecular layer, the Purkinje layer, and the granule cell layer.

The **molecular layer** is the outer layer and is made up of basket and stellate cells as well as parallel fibers, which are the axons of the granule cells. The extensive dendritic tree of the Purkinje cell extends into the molecular layer.

The **Purkinje layer** is the middle and most important layer of the cerebellar cortex. All of the inputs to the cerebellum are directed toward influencing the firing of Purkinje cells, and only axons of Purkinje cells leave the cerebellar cortex. A

single axon exits from each Purkinje cell and projects to one of the deep cerebellar nuclei or to vestibular nuclei of the brain stem.

The **granule cell layer** is the innermost layer of cerebellar cortex and contains Golgi cells, granule cells, and glomeruli. Each glomerulus is surrounded by a glial capsule and contains a granule cell and axons of Golgi cells, which synapse with granule cells. The granule cell is the only excitatory neuron within the cerebellar cortex. All other neurons in the cerebellar cortex, including Purkinje, Golgi, basket, and stellate cells, are inhibitory.

Table III-6-3. Cerebellum: Cell Types

Name	Target (Axon Termination)	Transmitter	Function
Purkinje cell	Deep cerebellar nuclei	GABA	Inhibitory*
Granule cell	Purkinje cell	Glutamate	Excitatory
Stellate cell	Purkinje cell	GABA	Inhibitory
Basket cell	Purkinje cell	GABA	Inhibitory
Golgi cell	Granule cell	GABA	Inhibitory

*Purkinje cells are the only outflow from the cerebellar cortex.

The internal white matter contains the deep cerebellar nuclei.

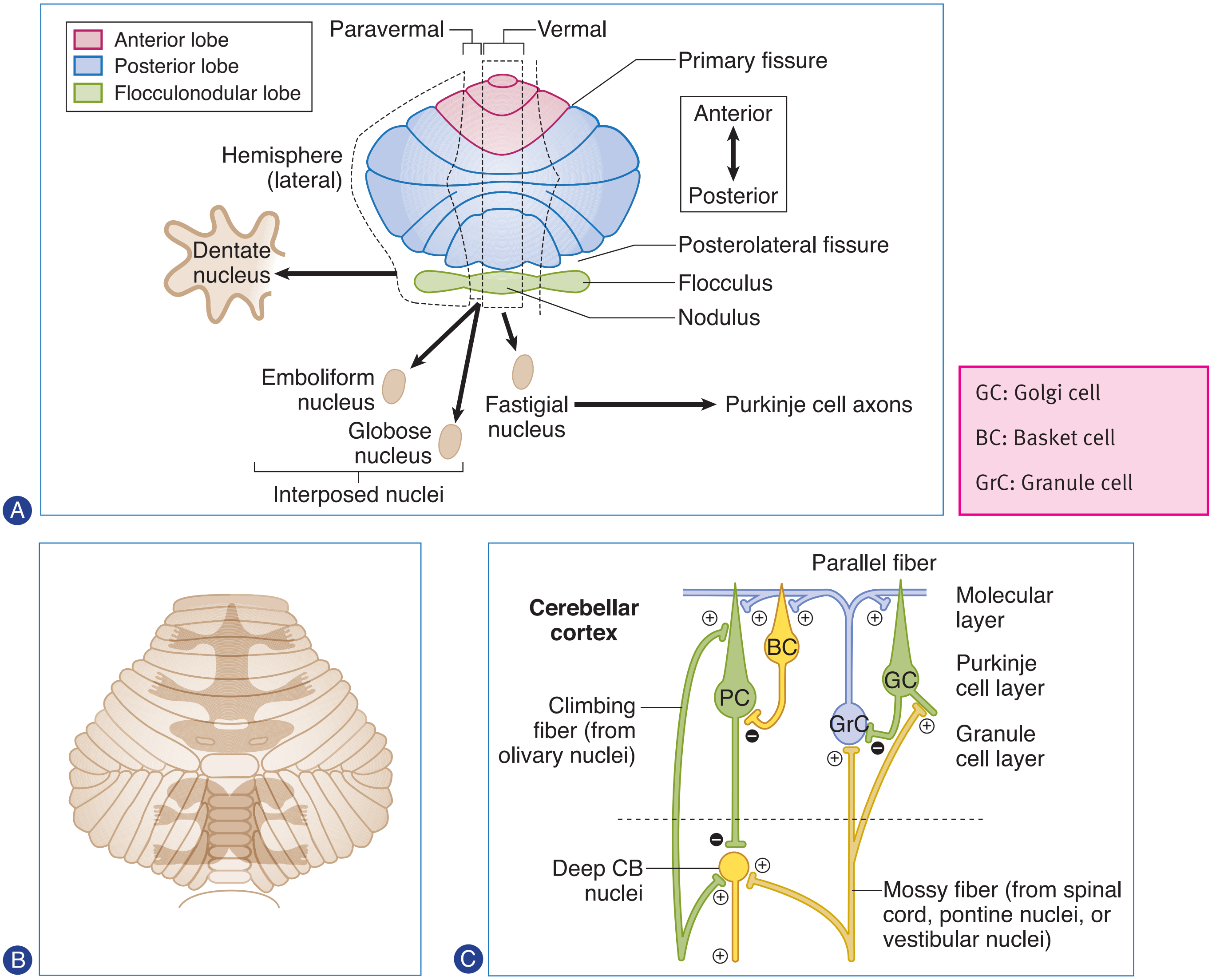


Figure III-6-2. Cerebellar Organization

(A) Parts of the cerebellar cortex and the deep cerebellar nuclei linked together by Purkinje cells
(B) Topographic arrangement of skeletal muscles controlled by parts of the cerebellum
(C) Cytology of the cerebellar cortex

From medial to lateral, the deep cerebellar nuclei in the internal white matter are the fastigial nucleus, interposed nuclei, and dentate nucleus.

Two kinds of excitatory input enter the cerebellum in the form of climbing fibers and mossy fibers. Both types influence the firing of deep cerebellar nuclei by axon collaterals.

Climbing fibers originate exclusively from the inferior olivary complex of nuclei on the contralateral side of the medulla. Climbing fibers provide a direct powerful monosynaptic excitatory input to Purkinje cells.

Mossy fibers represent the axons from all other sources of cerebellar input. Mossy fibers provide an indirect, more diffuse excitatory input to Purkinje cells.

All mossy fibers exert an excitatory effect on **granule cells**. Each granule cell sends its axon into the molecular layer, where it gives off collaterals at a 90-degree angle that run parallel to the cortical surface (i.e., parallel fibers). These granule cell axons stimulate the apical dendrites of the Purkinje cells. Golgi cells receive excitatory input from mossy fibers and from the parallel fibers of the granule cells. The Golgi cell in turn inhibits the granule cell, which activated it in the first place.

The **basket** and **stellate cells**, which also receive excitatory input from parallel fibers of granule cells, inhibit Purkinje cells.

CIRCUITRY

The basic cerebellar circuits begin with Purkinje cells that receive excitatory input directly from climbing fibers and from parallel fibers of granule cells.

Purkinje cell axons project to and inhibit the deep cerebellar nuclei or the vestibular nuclei in an orderly fashion.

- Purkinje cells in the flocculonodular lobe project to the lateral vestibular nucleus.
- Purkinje cells in the vermis project to the fastigial nuclei.
- Purkinje cells in the intermediate hemisphere primarily project to the interposed (globose and emboliform) nuclei.
- Purkinje cells in the lateral cerebellar hemisphere project to the dentate nucleus.

Dysfunction

- **Hemisphere lesions** → ipsilateral symptoms: **intention** tremor, dysmetria, dysdiadochokinesia, scanning dysarthria, nystagmus, hypotonia
- **Vermal lesions** → truncal ataxia

Major Pathway

Purkinje cells → deep cerebellar nucleus; dentate nucleus → contralateral VL → first-degree motor cortex → pontine nuclei → contralateral cerebellar cortex

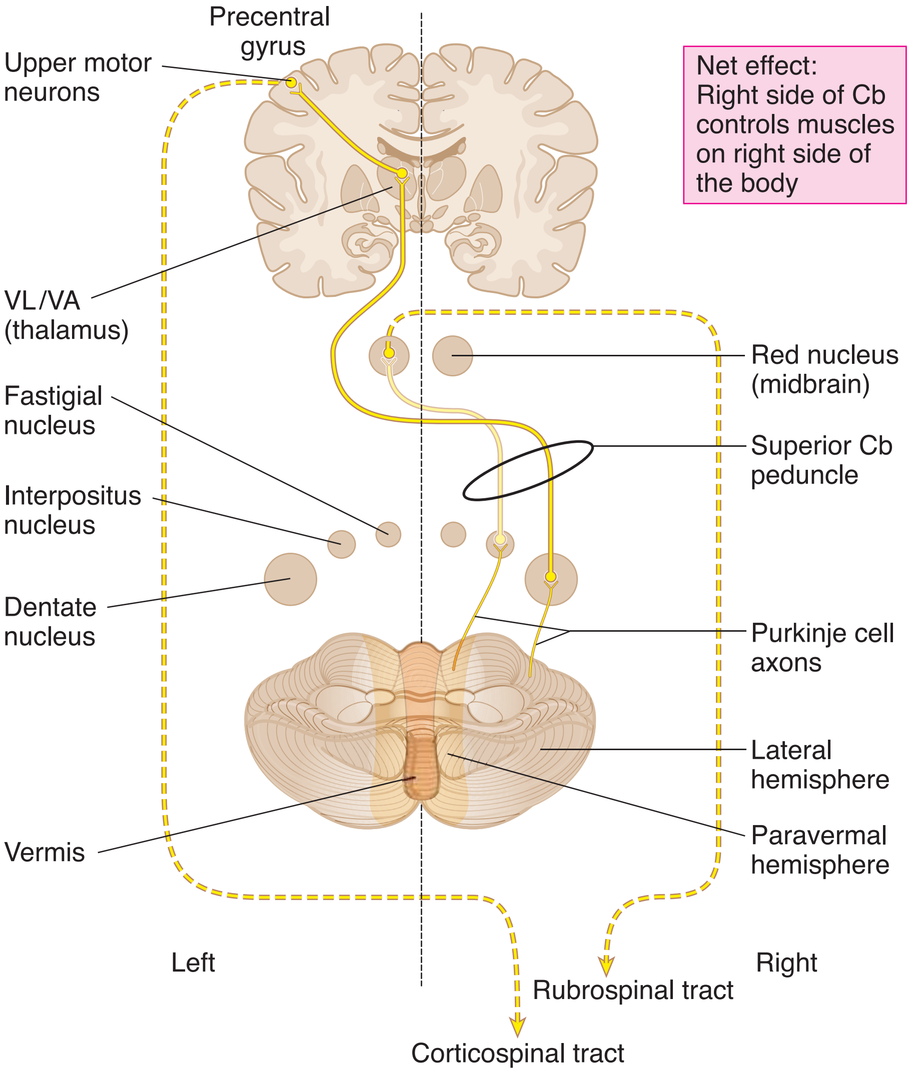


Figure III-6-3. Cerebellar Efferents

Table III-6-4. Major Efferents From the Cerebellum

Cerebellar Areas	Deep Cerebellar Nucleus	Efferents to:	Function
Vestibulocerebellum (flocculonodular lobe)	Fastigial nucleus	Vestibular nucleus	Elicit positional changes of eyes and trunk in response to movement of the head
Spinocerebellum (intermediate hemisphere)	Interpositus nucleus	Red nucleus Reticular formation	Influence LMNs via the reticulospinal and rubrospinal tracts to adjust posture and effect movement
Pontocerebellum (lateral hemispheres)	Dentate nucleus	Thalamus (VA, VL) then cortex	Influence on LMNs via the corticospinal tract, which effect voluntary movements, especially sequence and precision

Efferents from the deep cerebellar nuclei leave mainly through the SCP and influence all upper motoneurons. In particular, axons from the dentate and interposed nuclei leave through the SCP, cross the midline, and terminate in the ventrolateral (VL) nucleus of the thalamus.

The VL nucleus of the thalamus projects to primary motor cortex and influences the firing of corticospinal and corticobulbar neurons.

Axons from other deep cerebellar nuclei influence upper motoneurons in the red nucleus and in the reticular formation and vestibular nuclei.

Cerebellar Lesions

The hallmark of cerebellar dysfunction is a tremor with intended movement without paralysis or paresis. Symptoms associated with cerebellar lesions are expressed ipsilaterally because the major outflow of the cerebellum projects to the contralateral motor cortex, and then the corticospinal fibers cross on their way to the spinal cord. Thus, unilateral lesions of the cerebellum will result in a patient falling toward the side of the lesion.

Lesions that include the hemisphere

Lesions that include the hemisphere produce a number of dysfunctions, mostly involving distal musculature.

An intention tremor is seen when voluntary movements are performed. For example, if a patient with a cerebellar lesion is asked to pick up a penny, a slight tremor of the fingers is evident and increases as the penny is approached. The tremor is barely noticeable or is absent at rest.

Dysmetria (past pointing) is the inability to stop a movement at the proper place. The patient has difficulty performing the finger-to-nose test.

Dysdiadochokinesia (adiadochokinesia) is the reduced ability to perform alternating movements, such as pronation and supination of the forearm, at a moderately quick pace.

Scanning dysarthria is caused by asynergy of the muscles responsible for speech. In scanning dysarthria, patients divide words into syllables, thereby disrupting the melody of speech.

Gaze dysfunction occurs when the eyes try to fix on a point: They may pass it or stop too soon and then oscillate a few times before they settle on the target. A nystagmus may be present, particularly with acute cerebellar damage. The nystagmus is often coarse, with the fast component usually directed toward the involved cerebellar hemisphere.

Hypotonia usually occurs with an acute cerebellar insult that includes the deep cerebellar nuclei. The muscles feel flabby on palpation, and deep tendon reflexes are usually diminished.

Lesions to the vermal region

Vermal lesions result in difficulty maintaining posture, gait, or balance (an ataxic gait). Patients with vermal damage may be differentiated from those with a lesion of the dorsal columns by the Romberg sign. In cerebellar lesions, patients will sway or lose their balance with their eyes open; in dorsal column lesions, patients sway with their eyes closed.

Clinical Correlate

Anterior vermis lesions are usually the result of degeneration from alcohol abuse and are present with gait ataxia. Posterior vermis lesions result from medulloblastomas or ependymomas and present with truncal ataxia.

Basal Ganglia 7

Learning Objectives

❑ Solve problems concerning general features of the basal ganglia

GENERAL FEATURES

The basal ganglia initiate and provide gross control over skeletal muscle movements. The major components of the basal ganglia include:

- Striatum, which consists of the caudate nucleus and the putamen (telencephalon)
- External and internal segments of the globus pallidus (telencephalon)
- Substantia nigra (in midbrain)
- Subthalamic nucleus (in diencephalon)

Together with the cerebral cortex and the ventrolateral (VL) nucleus of the thalamus, these structures are interconnected to form 2 parallel but antagonistic circuits known as the direct and indirect basal ganglia pathways. Both pathways are driven by extensive inputs from large areas of cerebral cortex, and both project back to the motor cortex after a relay in the VL nucleus of the thalamus. Both pathways use a process known as "disinhibition" to mediate their effects, whereby one population of inhibitory neurons inhibits a second population of inhibitory neurons.

Direct Basal Ganglia Pathway

In the direct pathway, excitatory input from the cerebral cortex projects to striatal neurons in the caudate nucleus and putamen. Through disinhibition, activated inhibitory neurons in the striatum, which use γ-aminobutyric acid (GABA) as their neurotransmitter, project to and inhibit additional GABA neurons in the internal segment of the globus pallidus.

The GABA axons of the internal segment of the globus pallidus project to the thalamus (VL). Because their input to the thalamus is disinhibited, the thalamic input excites the motor cortex. The net effect of the disinhibition in the direct pathway results in an **increased** level of cortical excitation and the promotion of movement.

Indirect Basal Ganglia Pathway

In the indirect pathway, excitatory input from the cerebral cortex also projects to striatal neurons in the caudate nucleus and putamen. These inhibitory neurons in the striatum, which also use GABA as their neurotransmitter, project to and inhibit additional GABA neurons in the external segment of the globus pallidus.

The GABA axons of the external segment of the globus pallidus project to the subthalamic nucleus. Through disinhibition, the subthalamic nucleus excites inhibitory GABA neurons in the internal segment of the globus pallidus, which inhibits the thalamus. This decreases the level of cortical excitation, inhibiting movement. The net effect of the disinhibition in the indirect pathway results in a **decreased** level of cortical excitation, and a suppression of unwanted movement.

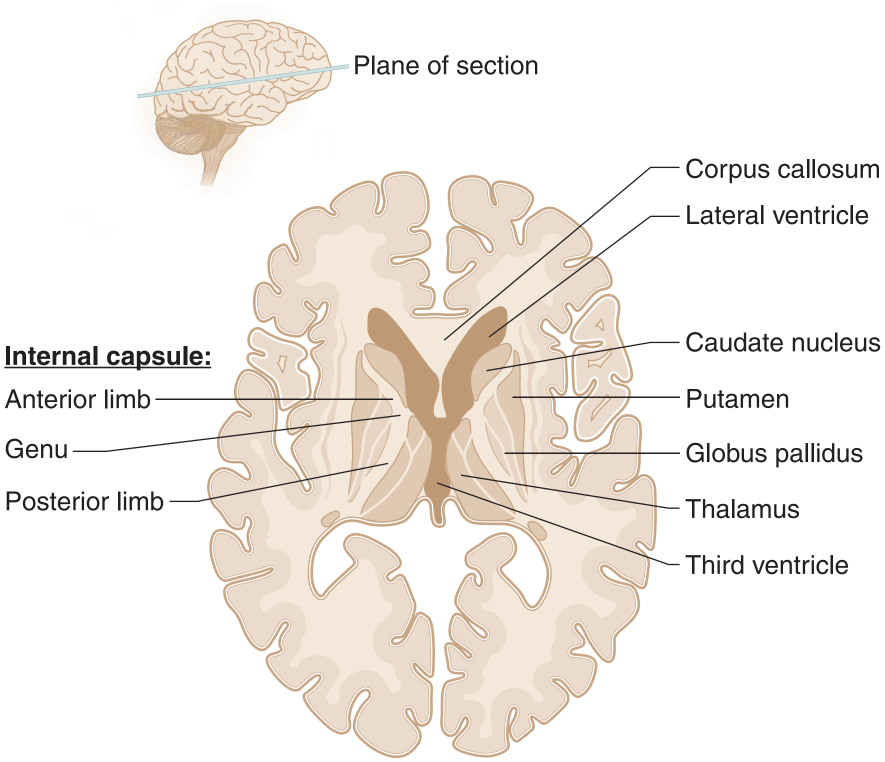


Figure III-7-1. Horizontal or Axial Section through Basal Ganglia

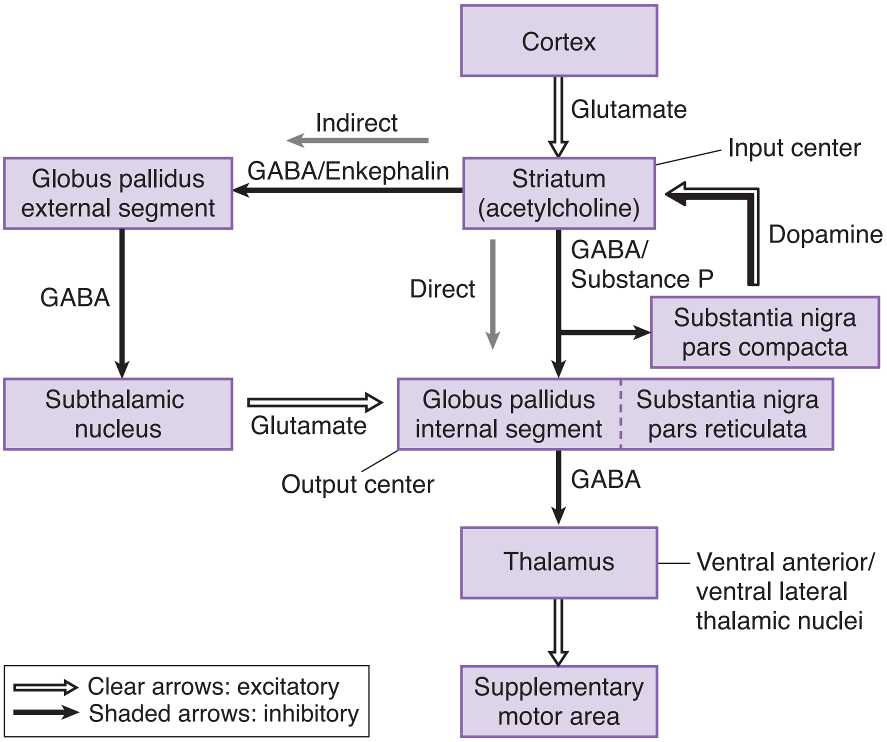


Figure III-7-2. Basal Ganglia Pathways

Note

Both basal ganglia pathways utilize 2 GABA neurons in series, and a "disinhibition."

Note

Dopamine drives the direct pathway; acetylcholine (ACh) drives the indirect pathway.

Dopamine and cholinergic effects

In addition to the GABA neurons, 2 other sources of chemically significant neurons enhance the effects of the direct or indirect pathways.

Dopaminergic neurons in the substantia nigra in the midbrain project to the striatum. The effect of dopamine excites or drives the direct pathway, increasing cortical excitation. Dopamine excites the direct pathway through D_1 receptors and inhibits the indirect pathway through D_2 receptors.

Cholinergic neurons found within the striatum have the opposite effect. Acetylcholine (Ach) drives the indirect pathway, decreasing cortical excitation.

Note

All basal ganglia connections are with ipsilateral cortex.

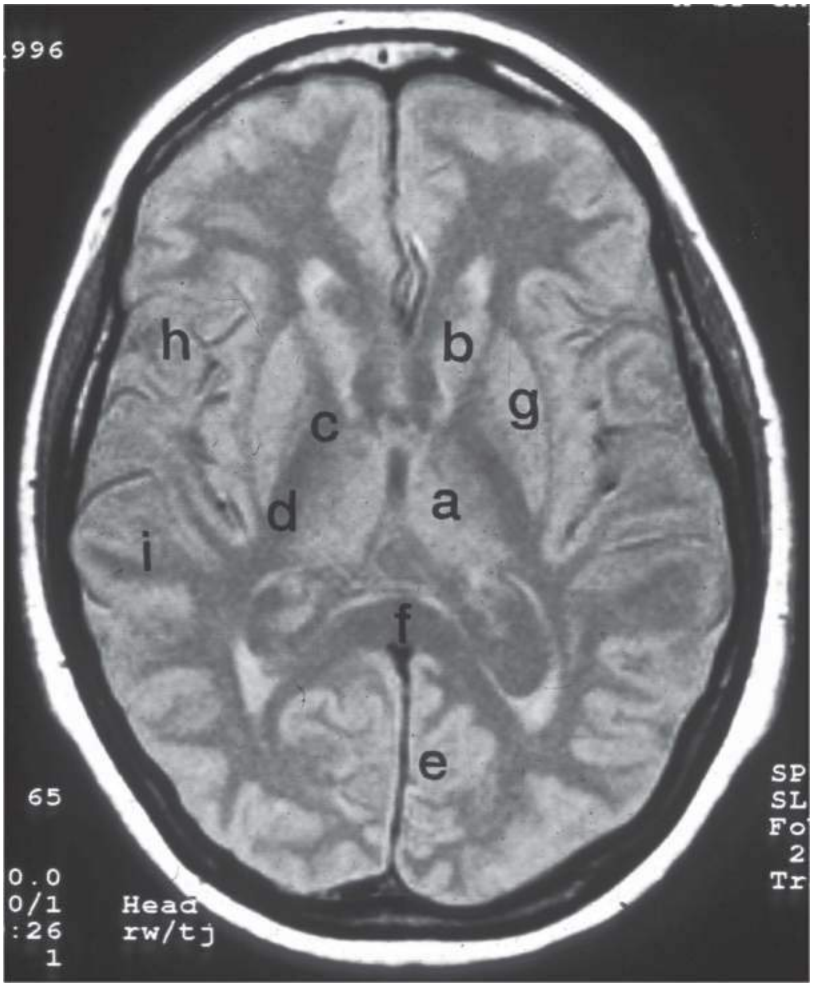


Figure III-7-3. MRI of Horizontal Section through Diencephalon, Basal Ganglia, and Cortex

(a) Thalamus (b) Head of Caudate Nucleus (c) Genu of Internal Capsule Containing Corticobulbar Axons (d) Posterior Limb of Internal Capsule (e) Primary Visual Cortex (f) Splenium of Corpus Callosum (g) Putamen (h) Broca's Motor Speech Area (i) Wernicke's Oral Comprehension Area

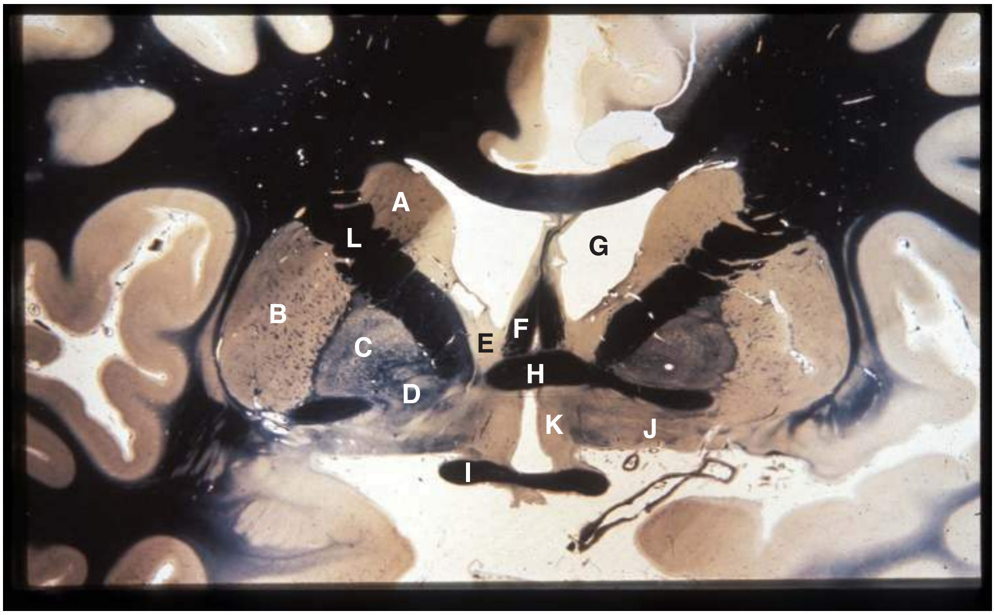


Figure III-7-4. Coronal Section through Basal Ganglia and Other Subcortical Structures

(A) caudate nucleus (B) putamen (C) globus pallidus external segment (D) globus pallidus internal segment (E) septal nuclei (F) fornix (G) lateral ventricle (H) anterior commissure (I) optic chiasm (J) basal nucleus of Meynert (K) preoptic hypothalamus (L) internal capsule, anterior limb

Table III-7-1. Diseases of the Basal Ganglia

Disease	Clinical Manifestations	Notes
Parkinson disease	Bradykinesia, cogwheel rigidity, pill-rolling (resting) tremor, shuffling gate, stooped posture, masked face, depression, dementia	**Loss of pigmented dopaminergic neurons from substantia nigra** **Lewy bodies:** intracytoplasmic eosinophilic inclusions, contain α-synuclein Known causes of parkinsonism: infections, vascular, and toxic insults (e.g., **MPTP**)
Disease	**Clinical Manifestations**	**Notes**
Huntington disease	Chorea (multiple, rapid, random movements), athetosis (slow, writhing movements), personality changes, dementia Onset: 20–40 years	**Degeneration** of GABAergic neurons in **neostriatum**, causing atrophy of head of caudate nucleus (and ventricular dilatation) **Autosomal dominant** **Unstable nucleotide repeat** on gene in chromosome 4, which codes for huntingtin protein Disease shows **anticipation** and **genomic imprinting** **Treatment:** antipsychotic agents, benzodiazepines, anticonvulsants
Wilson disease (hepatolenticular degeneration)	Tremor, asterixis, parkinsonian symptoms, chorea, neuropsychiatric symptoms; fatty change, hepatitis, or cirrhosis of liver, tremor may be "wing beating"	**Autosomal recessive defect in copper transport** Accumulation of copper in liver, brain, and eye (Descemet membrane, producing **Kayser-Fleischer ring**) Lesions in basal ganglia (especially putamen) **Treatment: penicillamine** (a chelator), zinc acetate (blocks absorption)
Hemiballism	Wild, flinging movements of limbs	Hemorrhagic destruction of **contralateral subthalamic nucleus** Hypertensive patients
Tourette syndrome	Motor tics and vocal tics (e.g., snorting, sniffing, uncontrolled and often obscene vocalizations), commonly associated with OCD and ADHD	**Treatment:** Antipsychotic agents

Abbreviations: ADHD, attention deficit hyperactivity disorder; MPTP, 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine; OCD, obsessive-compulsive disorder

Clinical Correlate

Lesions or diseases of the basal ganglia generally present with movement disorders, known as dyskinesias, and an involuntary tremor or tremor at rest.

Most basal ganglia disorders seem to preferentially affect either the direct or the indirect pathway, altering the balance between the two.

Lesions of the direct pathway result in an underactive cortex and hypokinetic disturbances in which there is a slowing or absence of spontaneous movements. The best-known disorder is caused by the degeneration of dopaminergic neurons of the substantia nigra in Parkinson disease. Because the cortex is underactive, Parkinson patients have problems initiating movements, combined with a reduction in the velocity and amplitude of the movements. The tremor at rest is the classic pill-rolling tremor seen in the fingers. Skeletal muscles in the upper limbs exhibit a cogwheel rigidity because of increased muscle tone. Patients also present with stooped posture, an expressionless face, and a festinating or accelerating gait during which individuals seem to chase their center of gravity. Strategies for Parkinson are L-dopa, a dopamine precursor that crosses the blood–brain barrier, anticholinergic drugs, which inhibit the effects of acetylcholine on the indirect pathway.

Other common disorders of the basal ganglia (chorea, athetosis, dystonia, tics) result from **lesions to parts of the indirect pathway**, which result in an overactive motor cortex. An overactive cortex produces hyperkinetic disturbances, expressed in numerous spontaneous movements. The involuntary tremors seen in these diseases range from being dancelike in chorea to ballistic with lesions to the subthalamic nucleus.

Chorea produces involuntary movements that are purposeless, quick jerks that may be superimposed on voluntary movements. Huntington chorea exhibits autosomal dominant inheritance (chromosome 4) and is characterized by severe degeneration of GABA neurons in the striatum. Patients suffer from athetoid movements, progressive dementia, and behavioral disorders. Sydenham chorea is a transient complication in some children with rheumatic fever.

Athetosis refers to slow, wormlike, involuntary movements that are most noticeable in the fingers and hands but may involve any muscle group. It is present in Huntington disease and may be observed in many diseases that involve the basal ganglia.

Dystonia refers to a slow, prolonged movement involving predominantly the truncal musculature. Dystonia often occurs with athetosis. Blepharospasm (contraction of the orbicularis oculi causing the eyelids to close), spasmodic torticollis (in which the head is pulled toward the shoulder), and writer's cramp (contraction of arm and hand muscles on attempting to write) are all examples of dystonic movements.

Hemiballismus results from a lesion of the subthalamic nucleus usually seen in hypertensive patients. Hemiballismus refers to a violent projectile movement of a limb and is typically observed in the upper limb contralateral to the involved subthalamic nucleus.

Tourette syndrome involves facial and vocal tics that progress to jerking movements of the limbs. It is frequently associated with explosive, vulgar speech.

Wilson disease results from an abnormality of copper metabolism, causing the accumulation of copper in the liver and basal ganglia. Personality changes, tremor, dystonia, and athetoid movements develop. Untreated patients usually succumb because of hepatic cirrhosis. A thin brown ring around the outer cornea, the Kayser-Fleischer ring, may be present and aid in the diagnosis.

8 Visual Pathways

Learning Objectives

- ❏ Use knowledge of eyeball and optic nerve
- ❏ Solve problems concerning visual reflexes
- ❏ Answer questions about lesions of the visual pathways

EYEBALL AND OPTIC NERVE

Light must pass through the cornea, aqueous humor, pupil, lens, and vitreous humor before reaching the retina. It must then pass through the layers of the retina to reach the photoreceptive layer of rods and cones. The outer segments of rods and cones transduce light energy from photons into membrane potentials. Photopigments in rods and cones absorb photons, and this causes a conformational change in the molecular structure of these pigments. This molecular alteration causes sodium channels to close, a hyperpolarization of the membranes of the rods and cones, and a reduction in the amount of neurotransmitter released. Thus, rods and cones release less neurotransmitter in the light and more neurotransmitter in the dark.

Rods and cones have synaptic contacts on bipolar cells that project to ganglion cells (Figure III-8-2). Axons from the ganglion cells converge at the optic disc to form the optic nerve, which enters the cranial cavity through the optic foramen. At the optic disc, these axons acquire a myelin sheath from the oligodendrocytes of the central nervous system (CNS).

Clinical Correlate

Vitamin A, necessary for retinal transduction, cannot be synthesized by humans. Dietary deficiency of vitamin A causes visual impairment resulting in night blindness.

Clinical Correlate

Decreased drainage into the canal of Schlemm is the most common cause of open-angle glaucoma.

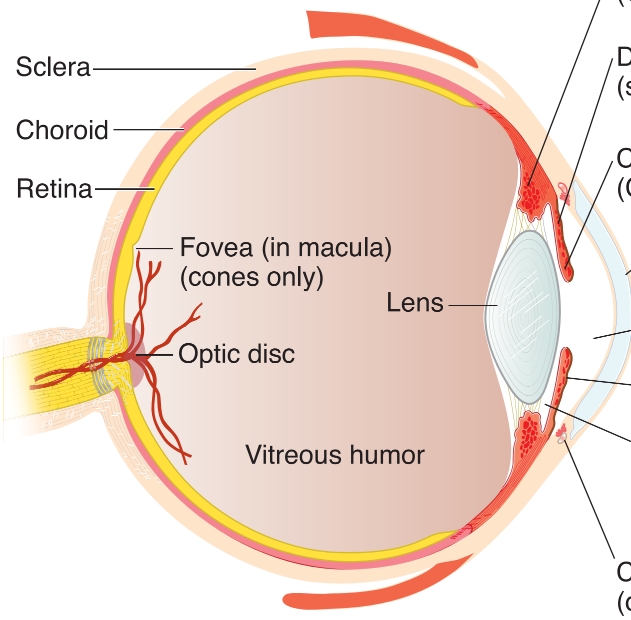


Figure III-8-1. The Eyeball

Open-Angle Glaucoma

A chronic condition (often with increased intraocular pressure [IOP]) due to decreased reabsorption of aqueous humor, leading to progressive (painless) visual loss and, if left untreated, blindness. IOP is a balance between fluid formation and its drainage from the globe.

Narrow-Angle Glaucoma

An acute (painful) or chronic (genetic) condition with increased IOP due to blockade of the canal of Schlemm. Emergency treatment prior to surgery often involves cholinomimetics, carbonic anhydrase inhibitors, and/or mannitol.

VISUAL REFLEXES

Pupillary Light Reflex

When light is directed into an eye, it stimulates retinal photoreceptors and results in impulses carried in the optic nerve to the pretectal area. Cells in the pretectal area send axons to the Edinger-Westphal nuclei on both sides.

The Edinger-Westphal nucleus is the parasympathetic nucleus of the oculomotor nerve and gives rise to preganglionic parasympathetic fibers that pass in the third cranial nerve to the ciliary ganglion. Because cells in the pretectal area supply both Edinger-Westphal nuclei, shining light into one eye results in constriction of both the ipsilateral pupil (direct light reflex) and contralateral pupil (consensual light reflex).

Accommodation-Convergence Reaction

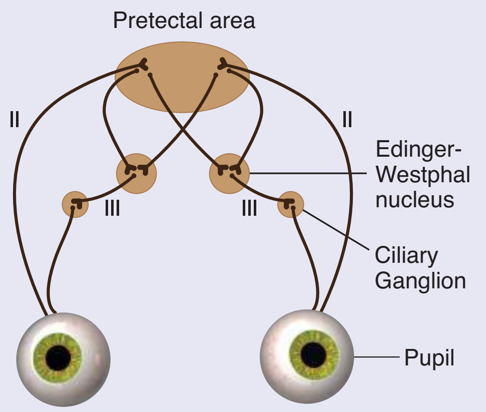
This reaction occurs when an individual attempts to focus on a nearby object after looking at a distant object. The oculomotor nerve carries the efferent fibers from the accommodation–convergence reaction, which consists of 3 components: accommodation, convergence, and pupillary constriction.

Accommodation refers to the reflex that increases the curvature of the lens needed for near vision. Preganglionic parasympathetic fibers arise in the Edinger-Westphal nucleus and pass via the oculomotor nerve to the ciliary ganglion. Postganglionic parasympathetic fibers from the ciliary ganglion supply the ciliary muscle. Contraction of this muscle relaxes the suspensory ligaments and allows the lens to increase its convexity (become more round). This increases the refractive index of the lens, permitting the image of a nearby object to focus on the retina.

Convergence results from contraction of both medial rectus muscles, which pull the eyes to look toward the nose. This allows the image of the near object to focus on the same part of the retina in each eye.

Pupillary constriction (miosis) results from contraction of the constrictor muscle of the iris. A smaller aperture gives the optic apparatus a greater depth of field. With Argyll Robertson pupils, both direct and consensual light reflexes are lost, but the accommodation–convergence reaction remains intact. This type of pupil is often seen in cases of neurosyphilis; however, it is sometimes seen in patients with multiple sclerosis, pineal tumors, or tabes dorsalis. The lesion site is believed to occur near the pretectal nuclei just rostral to the superior colliculi.

Table III-8-1. Pupillary Light Reflex Pathway



Afferent Limb: CN II	Light stimulates ganglion retinal cells → impulses travel up **CNII** which projects **bilaterally** to the **pretectal nuclei** (midbrain) The pretectal nucleus projects **bilaterally** → **Edinger-Westphal nuclei (CN III)**
Efferent Limb: CN III	Edinger-Westphal nucleus (preganglionic parasympathetic) → **ciliary ganglion** (postganglionic parasympathetic) → **pupillary sphincter muscle** → **miosis**

Because cells in the pretectal area supply the Edinger-Westphal nuclei bilaterally, shining light in one eye → constriction in the ipsilateral pupil (direct light reflex) and the contralateral pupil (consensual light reflex).

Because this reflex does not involve the visual cortex, a person who is cortically blind can still have this reflex.

The eye is predominantly innervated by the parasympathetic nervous system. Therefore, application of muscarinic antagonists or ganglionic blockers has a large effect by blocking the parasympathetic nervous system.

Table III-8-2. Pharmacology of the Eye

Structure	Predominant Receptor	Receptor Stimulation	Receptor Blockade
Pupillary sphincter ms. (iris)	M_3 receptor (PANS)	Contraction → miosis	Relaxation → mydriasis
Radial dilator ms. (iris)	α receptor (SANS)	Contraction → mydriasis	Relaxation → miosis
Ciliary ms.	M_3 receptor (PANS)	Contraction → accommodation for near vision	Relaxation → focus for far vision
Ciliary body epithelium	β receptor (SANS)	Secretion of aqueous humor	Decreased aqueous humor production

Abbreviations: ms., muscle; PANS, parasympathetic nervous system; SANS, sympathetic nervous system

Table III-8-3. Accommodation-Convergence Reaction

When an individual focuses on a nearby object after looking at a distant object, 3 events occur: 1. **Accommodation** 2. **Convergence** 3. **Pupillary constriction (miosis)** In general, stimuli from light → visual cortex → superior colliculus and pretectal nucleus → Edinger-Westphal nucleus (1, 3) and oculomotor nucleus (2).
Accommodation: Parasympathetic fibers contract the ciliary muscle, which relaxes suspensory ligaments, allowing the lens to increase its convexity (become more round). This increases the refractive index of the lens, thereby focusing a nearby object on the retina.
Convergence: Both medial rectus muscles contract, adducting both eyes.
Pupillary constriction: Parasympathetic fibers contract the pupillary sphincter muscle → miosis.

Table III-8-4. Clinical Correlates

	Pupillary Abnormalities
Argyll Robertson pupil (pupillary light-near dissociation)	• No direct or consensual light reflex; accommodation-convergence intact • Seen in **neurosyphilis,** diabetes
Relative afferent (Marcus Gunn) pupil	• Lesion of afferent limb of pupillary light reflex; diagnosis made with swinging flashlight • Shine light in Marcus Gunn pupil → pupils do not constrict fully • Shine light in normal eye → pupils constrict fully • Shine light immediately again in affected eye → apparent dilation of both pupils because stimulus carried through that CN II is weaker; seen in multiple sclerosis
Horner syndrome	• Caused by a lesion of the oculosympathetic pathway; syndrome consists of miosis, ptosis, apparent enophthalmos, and hemianhidrosis
Adie pupil	• Dilated pupil that reacts sluggishly to light, but better to accommodation; often seen in women and often associated with loss of knee jerks. Ciliary ganglion lesion
Transtentorial (uncal) herniation	• Increased intracranial pressure → leads to uncal herniation → CN III compression → fixed and dilated pupil, “down-and-out” eye, ptosis

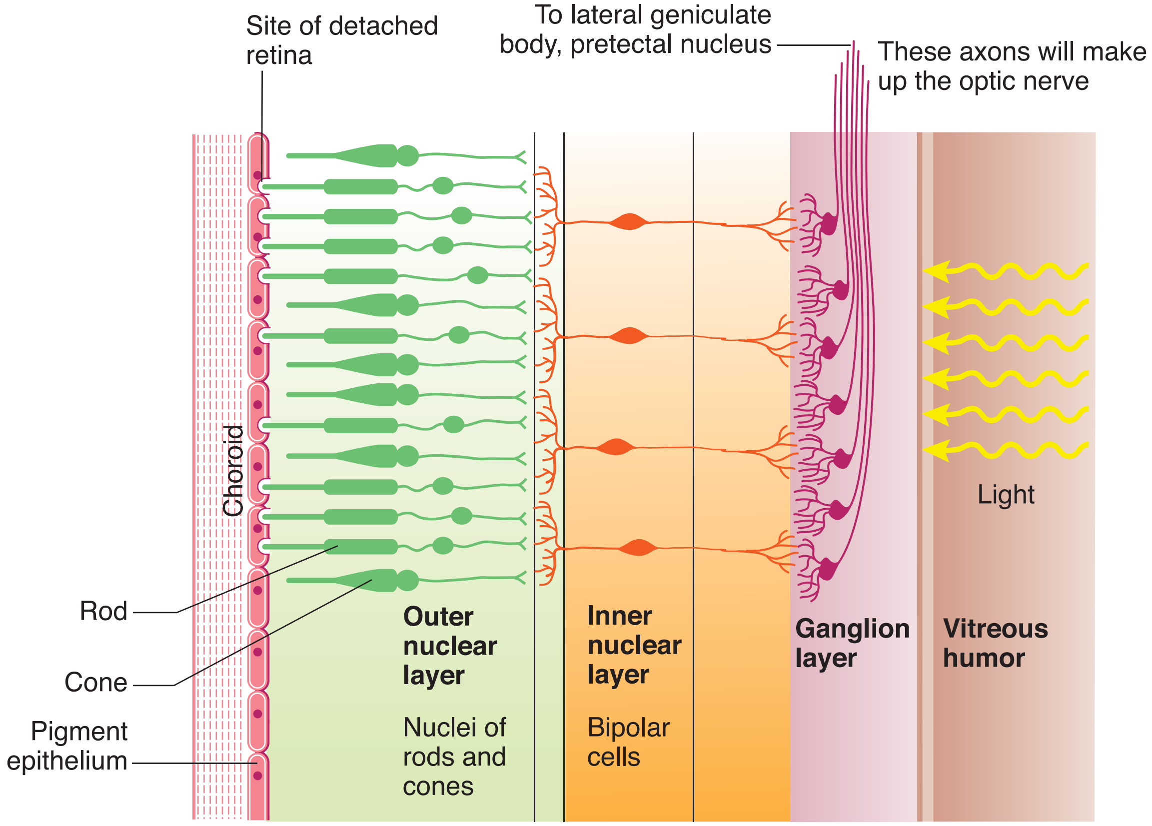


Figure III-8-2. Retina

Note

Photoreceptor Rods: 1 kind

- Achromatic
- Low-light sensitive
- Night vision, motion

Cones: 3 kinds

- Red, green, blue
- Chromatic
- Bright light sensitive
- Object recognition

At the optic chiasm, 60% of the optic nerve fibers from the nasal half of each retina cross and project into the contralateral optic tract (Figure III-8-3). Fibers from the temporal retina do not cross at the chiasm and instead pass into the ipsilateral optic tract. The optic tract contains remixed optic nerve fibers from the temporal part of the ipsilateral retina and fibers from the nasal part of the contralateral retina. Because the eye inverts images like a camera, in reality each nasal retina receives information from a temporal hemifield, and each temporal retina receives information from a nasal hemifield. Most fibers in the optic tract project to the lateral geniculate nucleus. Optic tract fibers also project to the superior colliculi for reflex gaze, to the pretectal area for the light reflex, and to the suprachiasmatic nucleus of the hypothalamus for circadian rhythms.

Visual Field Defects

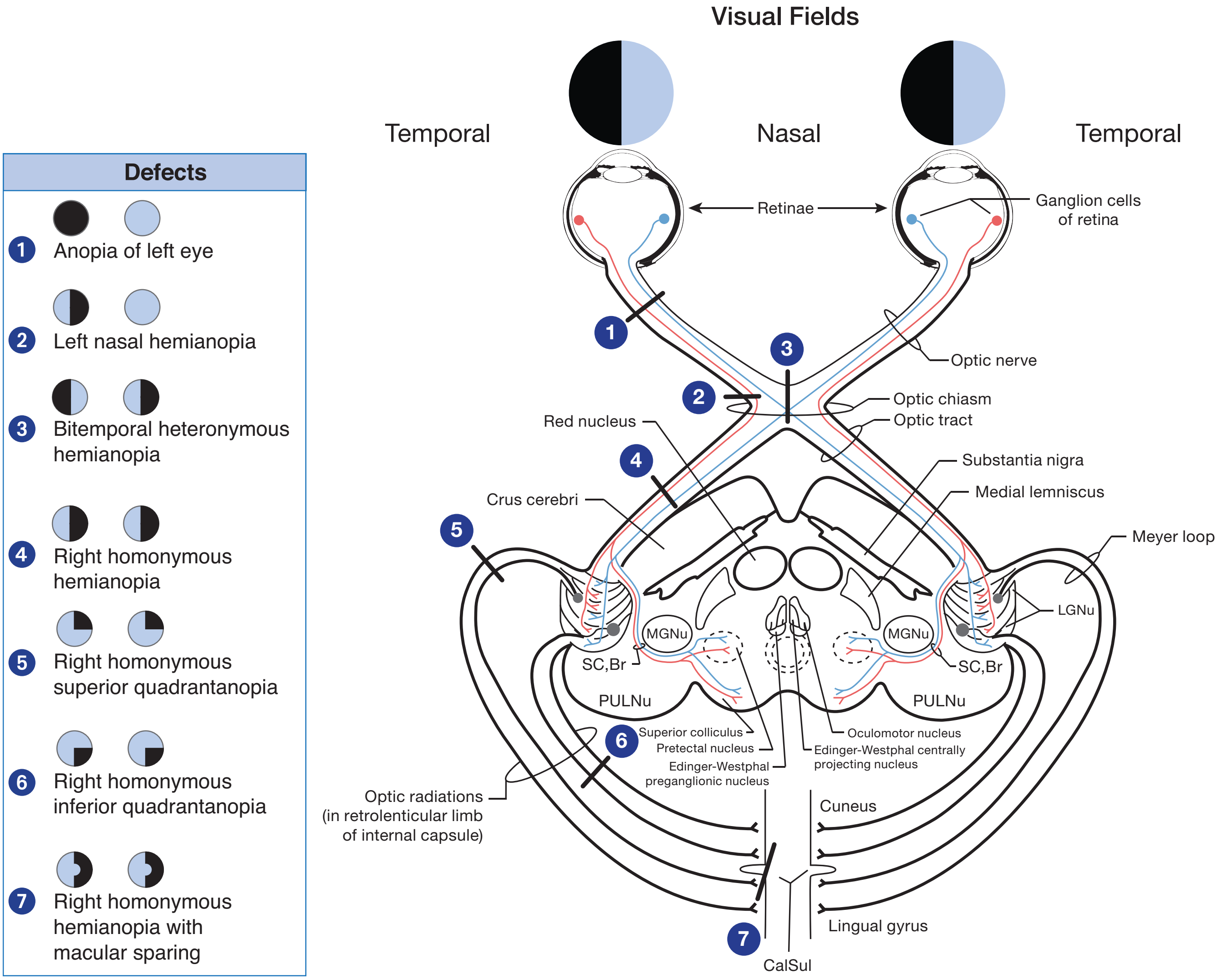


Figure III-8-3. Visual Pathways

1, 2 Optic nerve, 3 Chiasm, 4 Tract

Note

Visual information from **lower** retina courses in **lateral** fibers forming Meyer's **loop**, which projects to the lingual gyrus.

Clinical Correlate

Some Causes of Lesions

1. Optic neuritis, central retinal artery occlusion
2. Internal carotid artery aneurysm
3. Pituitary adenoma (begins as superior quadrantanopia) Craniopharyngioma (begins as inferior quadrantanopia)
4. Vascular
5. Middle cerebral artery (MCA) occlusion

6,7. Posterior cerebral artery occlusion Macula is spared in 7 due to collateral blood supply from MCA.

Note

Lesions to the visual radiations are more common than lesions to the optic tract.

Notes

Like a camera, the lens inverts the image of the visual field, so the nasal retina receives information from the temporal visual field, and the temporal retina receives information from the nasal visual field.

At the **optic chiasm**, optic nerve fibers from the nasal half of each retina cross and project to the contralateral optic tract.

Clinical Correlate

Unilateral optic nerve lesions are seen in MS, where there is an immune-related inflammatory demyelination of the nerve. The lesion typically presents with a central scotoma due to involvement of the deep fibers in the nerve from the macula.

Most fibers from the **optic tract** project to the **lateral geniculate body (LGB)**; some also project to the pretectal area (light reflex), the superior colliculi (reflex gaze), and the suprachiasmatic nuclei (circadian rhythm). The LGB projects to the **primary visual cortex** (striate cortex, Brodmann area 17) of the occipital lobe via the optic radiations.

- Visual information from the lower retina (upper contralateral visual field) → temporal lobe (**Meyer loop**) → **lingual gyrus**
- Visual information from the upper retina (lower contralateral visual field) → parietal lobe → **cuneus gyrus**

The lateral geniculate body (LGB) is a laminated structure that receives input from the optic tract and gives rise to axons that terminate on cells in the primary visual cortex (striate cortex, Brodmann area 17) of the occipital lobe. The LGB laminae maintain a segregation of inputs from the ipsilateral and contralateral retina.

The axons from the LGB that project to the striate cortex are known asoptic radiations, visual radiations, or the geniculocalcarine tract. The calcarine sulcus divides the striate cortex (primary visual cortex or Brodmann area 17) into the cuneus and the lingual gyri. The cuneus gyrus, which lies on the superior bank of the calcarine cortex, receives the medial fibers of the visual radiations. The lingual gyrus, which lies on the inferior bank of the calcarine cortex, receives the lateral fibers of the visual radiation. The medial fibers coursing in the visual radiations, which carry input from the upper retina (i.e., the lower contralateral visual field), pass from the LGB directly through the parietal lobe to reach the cuneus gyrus. Significantly, the lateral fibers coursing in the visual radiations, which carry input from the lower retina (i.e., the upper contralateral visual field), take a circuitous route from the LGB through Meyer loop anteriorly into the temporal lobe. The fibers of Meyer loop then turn posteriorly and course through the parietal lobe to reach the lingual gyrus in the striate cortex.

LESIONS OF THE VISUAL PATHWAYS

Lesions of the retina that include destruction of the macula produce a central scotoma. The macula is quite sensitive to intense light, trauma, aging, and neurotoxins.

Lesions of an optic nerve produce blindness (anopsia) in that eye and a loss of the sensory limb of the light reflex. The pupil of the affected eye constricts when light is shined into the opposite eye (consensual light reflex) but not when light is shined into the blinded eye (absence of direct light reflex).

Compression of the optic chiasm, often the result of a pituitary tumor or meningioma, results in a loss of peripheral vision in both temporal fields because the crossing fibers from each nasal retina are damaged. The resulting visual field defect is called a bitemporal heteronymous hemianopia.

All lesions past the chiasm produce contralateral defects. Lesions of the optic tract result in a loss of visual input from the contralateral visual field. For example, a lesion of the right optic tract results in a loss of input from the left visual field. This is called a homonymous hemianopia; in this example, a left homonymous hemianopia.

Lesions of the visual radiations are more common than lesions to the optic tract or lateral geniculate body and produce visual field defects (a contralateral homonymous hemianopia) similar to those of the optic tract if all fibers are involved.

Lesions restricted to the lateral fibers in Meyer loop, usually in the temporal lobe, result in a loss of visual input from the contralateral upper quarter of the visual field. For example, a lesion of the temporal fibers in the **right** visual radiation results in loss of visual input from the upper left quarter of the field (a **left** superior quadrantanopia).

Lesions restricted to the medial fibers in the visual radiation in the parietal lobe result in a loss of visual input from the contralateral lower quarter of the field (an inferior quadrantanopia).

Lesions inside the primary visual cortex are equivalent to those of the visual radiations, resulting in a contralateral homonymous hemianopsia, except that macular (central) vision is spared.

Lesions of the cuneus gyrus are equivalent to lesions restricted to the parietal fibers of the visual radiation, with macular sparing.

Lesions of the lingula are similar to lesions of the Meyer's loop fibers except for the presence of macular sparing. The pupillary light reflex is spared in lesions of the radiations or inside visual cortex because fibers of the pupillary light reflex leave the optic tracts to terminate in the pretectal area. The combination of blindness with intact pupillary reflexes is termed cortical blindness.

Diencephalon 9

Learning Objectives

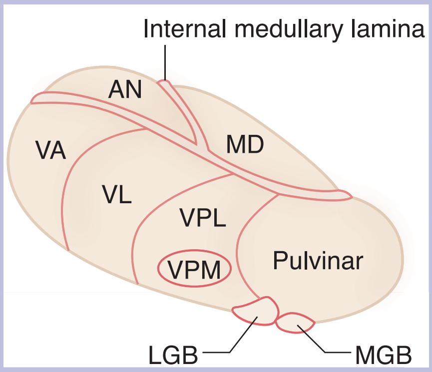
- ❏ Interpret scenarios on thalamus
- ❏ Demonstrate understanding of hypothalamus
- ❏ Use knowledge of epithalamus

DIENCEPHALON

The diencephalon can be divided into 4 parts: the thalamus, the hypothalamus, the epithalamus, and the subthalamus.

Table III-9-1. Thalamus

Thalamus—serves as a major sensory relay for information that ultimately reaches the neocortex. Motor control areas (basal ganglia, cerebellum) also synapse in the thalamus before reaching the cortex. Other nuclei regulate states of consciousness.



Thalamic Nuclei	Input	Output
VPL	Sensory from **body and limbs**	Somatosensory cortex
VPM	Sensory from **face, taste**	Somatosensory cortex
VA/VL	**Motor** info from BG, cerebellum	Motor cortices
LGB	**Visual** from optic tract	First-degree visual cortex
MGB	**Auditory** from inferior colliculus	First-degree auditory cortex
AN	Mamillary nucleus (via mammillothalamic tract)	Cingulate gyrus (part of **Papez** circuit)
MD	(Dorsomedial nucleus). Involved in **memory** Damaged in **Wernicke-Korsakoff** syndrome	
Pulvinar	Helps integrate somesthetic, visual, and auditory input	
Midline/intralaminar	Involved in **arousal**	

Abbreviations: AN, anterior nuclear group; BG, basal ganglia; LGB, lateral geniculate body; MD, mediodorsal nucleus; MGB, medial geniculate body; VA, ventral anterior nucleus; VL, ventral lateral nucleus; VPL, ventroposterolateral nucleus; VPM, ventroposteromedial nucleus

Thalamus

The thalamus serves as the major sensory relay for the ascending tactile, visual, auditory, and gustatory information that ultimately reaches the neocortex. Motor control areas such as the basal ganglia and cerebellum also synapse in thalamic nuclei before they reach their cortical destinations. Other nuclei participate in the regulation of states of consciousness.

Clinical Correlate

Thiamine deficiency in alcoholics results in degeneration of the dorsomedial nucleus of thalamus and the mammillary bodies, hippocampus, and vermis of the cerebellum.

Major Thalamic Nuclei and Their Inputs and Outputs

Anterior nuclear group (part of the Papez circuit of limbic system)

Input is from the mammillary bodies via the mammillothalamic tract and from the cingulate gyrus; output is to the cingulate gyrus via the anterior limb of the internal capsule.

Medial nuclear group (part of limbic system)

Input is from the amygdala, prefrontal cortex, and temporal lobe; output is to the prefrontal cortex and cingulate gyrus. The most important nucleus is the dorsomedial nucleus.

Clinical Correlate

Thalamic pain syndrome affects the ventral nuclear group. Patients present with burning, aching pain in contralateral limbs or body. Involvement of the dorsal column-medial lemniscal part of VPL increases the sensitivity to pain and presents as contralateral loss of vibratory sense and gait ataxia. Thalamic pain syndrome is resistant to analgesic medications.

Ventral nuclear group

Motor Nuclei

Ventral anterior nucleus (VA): Input to VA is from the globus pallidus, substantia nigra. Output is to the premotor and primary motor cortex.

Ventral lateral nucleus (VL): Input to VL is mainly from the globus pallidus and the dentate nucleus of the cerebellum. Output is to the primary motor cortex (Brodmann area 4).

Sensory Nuclei

Ventral posterolateral (VPL) nucleus: Input to VPL conveying somatosensory and nociceptive information ascends in the medial lemniscus and spinothalamic tract. Output is to primary somatosensory cortex (Brodmann areas 3, 1, and 2) of the parietal lobe.

Ventral posteromedial (VPM) nucleus: Input to VPM is from the ascending trigeminal and taste pathways. Output is to primary somatosensory cortex (Brodmann areas 3, 1, and 2) of the parietal lobe.

Medial geniculate body (nucleus): Input is from auditory information that ascends from the inferior colliculus. Output is to primary auditory cortex.

Lateral geniculate body (nucleus): Input is from the optic tract. Output is in the form of the geniculocalcarine or visual radiations that project to the primary visual (striate) cortex in the occipital lobe.

Midline and Intralaminar Nuclei

Midline and intralaminar nuclei receive input from the brain-stem reticular formation, and from the spinothalamic tract. Intralaminar nuclei send pain information to the cingulate gyrus. These nuclei appear to be important in mediating desynchronization of the EEG during behavioral arousal.

Hypothalamus

The hypothalamus is composed of numerous nuclei that have afferent and efferent connections with widespread regions of the nervous system, including the pituitary gland, the autonomic system, and the limbic system (Figure III-9-1).

Table III-9-2. Hypothalamus, Epithalamus, Subthalamus

Hypothalamus— helps maintain homeostasis; has roles in the autonomic, endocrine, and limbic systems	
Hypothalamic Nuclei	Functions and Lesions
Lateral hypothalamic	**Feeding center;** lesion → starvation
Ventromedial	**Satiety center;** lesion → hyperphagia, obesity, savage behavior
Suprachiasmatic	Regulates circadian rhythms, receives direct retinal input
Supraoptic and paraventricular	Synthesizes **ADH** and **oxytocin; regulates water balance** Lesion → **diabetes insipidus**, characterized by polydipsia and polyuria
Mamillary body	Input from hippocampus; damaged in Wernicke encephalopathy
Arcuate	Produces hypothalamic releasing and inhibiting factors and gives rise to tuberohypophysial tract Has neurons that produce dopamine (prolactin-inhibiting factor)
Anterior region	**Temperature regulation;** lesion → hyperthermia Stimulates the parasympathetic nervous system
Posterior region	**Temperature regulation;** lesion → poikilothermia (inability to thermoregulate) Stimulates sympathetic nervous system
Preoptic area	Regulates release of gonotrophic hormones; contains sexually dimorphic nucleus Lesion before puberty → arrested sexual development; lesion after puberty → amenorrhea or impotence
Dorsomedial	Stimulation → savage behavior
Epithalamus— Consists of pineal body and habenular nuclei. The **pineal body** secretes melatonin with a **circadian rhythm**.	
Subthalamus— The **subthalamic nucleus** is involved in **basal ganglia** circuitry. Lesion → **hemiballismus** (contralateral flinging movements of one or both extremities)	

Abbreviation: ADH, antidiuretic hormone

Clinical Correlate

Dopaminergic projections from the arcuate nuclei inhibit prolactin secretion from the anterior pituitary. Lesions result in galactorrhea (milk discharge) and amenorrhea.

Clinical Correlate

Lesions of the mammillary bodies occur in **Korsakoff syndrome** and are usually associated with thiamine deficiency associated with chronic alcoholism. Korsakoff syndrome results in both anterograde and retrograde amnesia with confabulations.

Major Hypothalamic Regions or Zones, and Their Nuclei

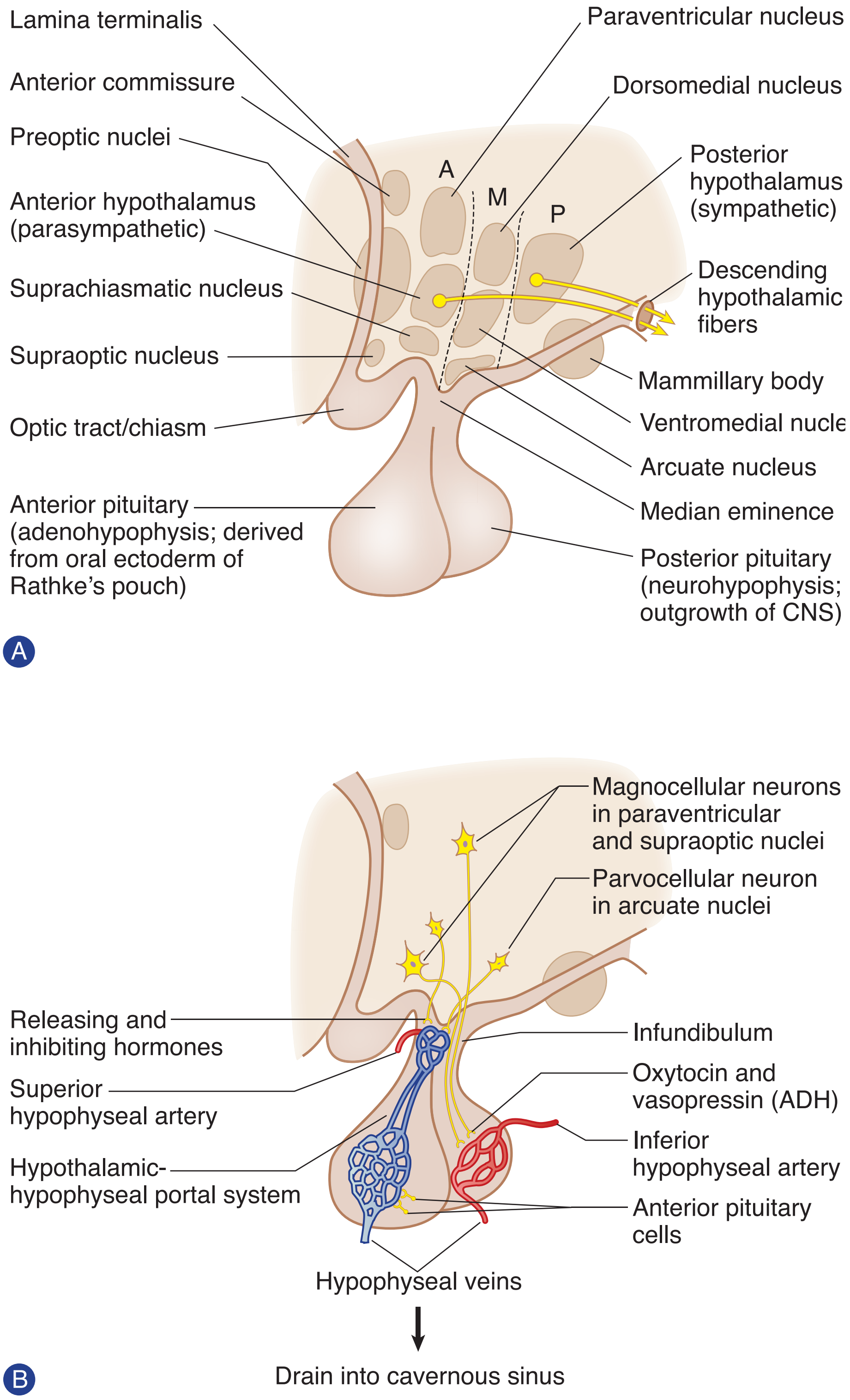


Figure III-9-1. (A) Organization of the Hypothalamus (Sagittal Section) (B) Secretory Mechanisms of the Adeno- and Neuro-Hypophysis

Anterior region

The **paraventricular and supraoptic nuclei** synthesize the neuropeptides antidiuretic hormone (ADH) and oxytocin. Axons arising from these nuclei leave the hypothalamus and course in the supraopticohypophysial tract, which carries neurosecretory granules to the posterior pituitary gland, where they are released into capillaries. Lesions of the supraoptic nuclei lead to diabetes insipidus, which is characterized by polydipsia (excess water consumption) and polyuria (excess urination).

Visual input from the retina by way of the optic tract terminates in the **suprachiasmatic nucleus**. This information helps set certain body rhythms to the 24-hour light-dark cycle (circadian rhythms).

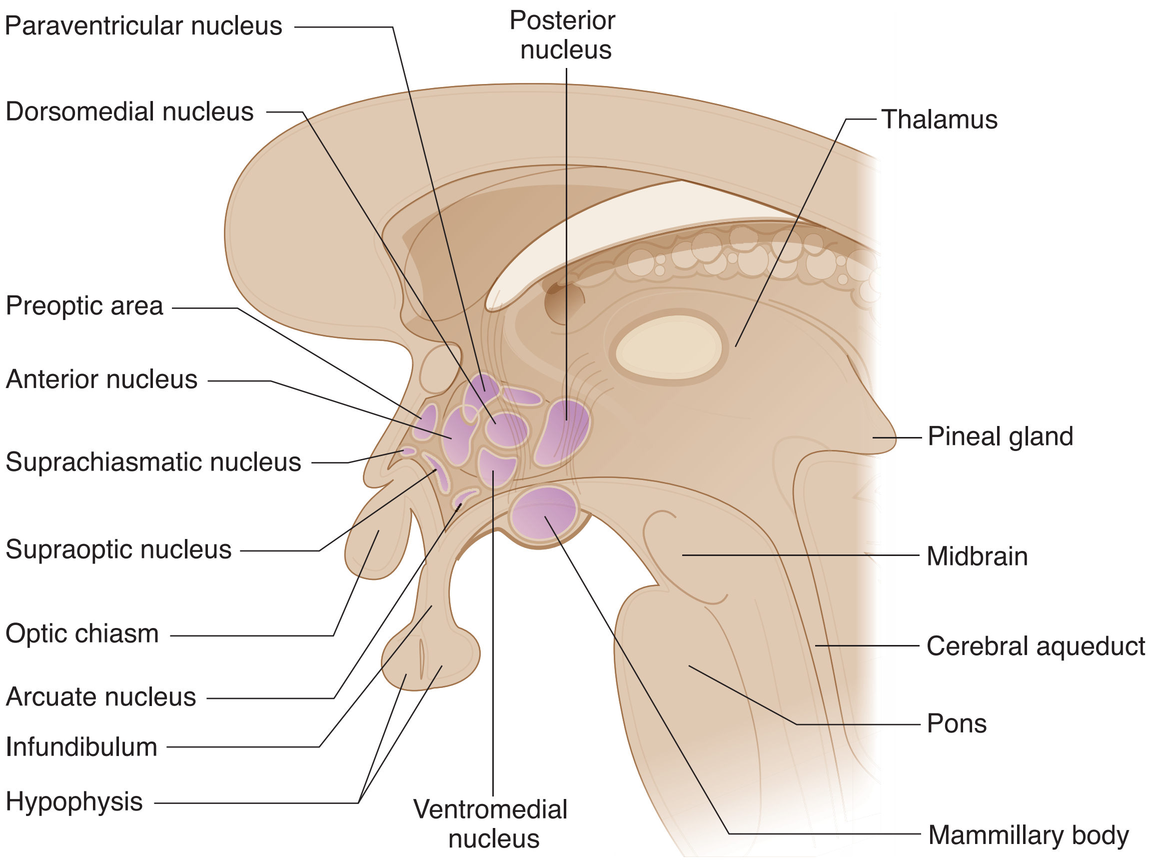


Figure III-9-2. The Hypothalamic Nuclei

Tuberal region

Cells in the **arcuate nucleus** produce releasing hormones and inhibitory factors, which enter capillaries in the tuberoinfundibular tract and pass through the hypophyseal-portal veins to reach the secondary capillary plexus in the anterior pituitary gland. Releasing hormones and inhibitory factors influence the secretory activity of the acidophils and basophils in the anterior pituitary. (See Histology section.)

The **ventromedial hypothalamus** is a satiety center and regulates food intake. Lesions of the ventromedial hypothalamus result in obesity.

Posterior region

The mammillary nuclei are located in the **mammillary bodies** and are part of the limbic system. The mammillothalamic tract originates in the mammillary nuclei and terminates in the anterior nuclear group of the thalamus.

Anterior hypothalamic zone

The anterior hypothalamic zone senses an elevation of body temperature and mediates the response to dissipate heat. Lesions of the anterior hypothalamus lead to hyperthermia.

Posterior hypothalamic zone

The posterior hypothalamic zone senses a decrease of body temperature and mediates the conservation of heat. Lesions of the posterior hypothalamus lead to poikilothermy (i.e., cold-blooded organisms). An individual with a lesion of the posterior hypothalamus has a body temperature that varies with the environmental temperature.

Lateral hypothalamic zone

The lateral hypothalamic zone is a feeding center; lesions of the lateral hypothalamus produce severe aphagia.

Preoptic area

The preoptic area is sensitive to androgens and estrogens, whereas other areas influence the production of sex hormones through their regulation of the anterior pituitary. Before puberty, hypothalamic lesions here may arrest sexual development.

After puberty, hypothalamic lesions in this area may result in amenorrhea or impotence.

Epithalamus

The epithalamus is the part of the diencephalon located in the region of the posterior commissure which consists of the pineal body and the habenular nuclei.

- The pineal body is a small, highly vascularized structure situated above the posterior commissure and attached by a stalk to the roof of the third ventricle. It contains pinealocytes and glial cells but no neurons. Pinealocytes synthesize melatonin, serotonin, and cholecystokinin.
- The pineal gland plays a role in growth, development, and the regulation of circadian rhythms.
- Environmental light regulates the activity of the pineal gland through a retinal–suprachiasmatic– pineal pathway.
- The subthalamus is reviewed with the basal ganglia.

Clinical Correlate

- In young males, pineal lesions may cause **precocious puberty**.
- **Pineal tumors** may cause obstruction of CSF flow and increased intracranial pressure. Compression of the upper midbrain and pretectal area by a pineal tumor results in Parinaud syndrome, in which there is impairment of conjugate vertical gaze and pupillary reflex abnormalities.

Cerebral Cortex 10

Learning Objectives

- ❑ Answer questions about general features
- ❑ Solve problems concerning language and the dominant hemisphere
- ❑ Solve problems concerning blood supply

GENERAL FEATURES

The surface of the cerebral cortex is highly convoluted with the bulges or eminences, referred to as gyri; and the spaces separating the gyri, called sulci. Lobes of the cerebrum are divided according to prominent gyri and sulci that are fairly constant in humans.

Two prominent sulci on the lateral surface are key to understanding the divisions of the hemispheres. The lateral fissure (of Sylvius) separates the frontal and temporal lobes rostrally; further posteriorly, it partially separates the parietal and the temporal lobes. The central sulcus (of Rolando) is situated roughly perpendicular to the lateral fissure. The central sulcus separates the frontal and the parietal lobes. The occipital lobe extends posteriorly from the temporal and parietal lobes, but its boundaries on the lateral aspect of the hemisphere are indistinct. On the medial aspect of the hemisphere, the frontal and parietal lobes are separated by a cingulate sulcus from the cingulate gyrus. The cingulate is part of an artificial limbic lobe. Posteriorly, the parieto-occipital sulcus separates the parietal lobe from the occipital lobe. The calcarine sulcus divides the occipital lobe horizontally into a superior cuneus and an inferior lingual gyrus.

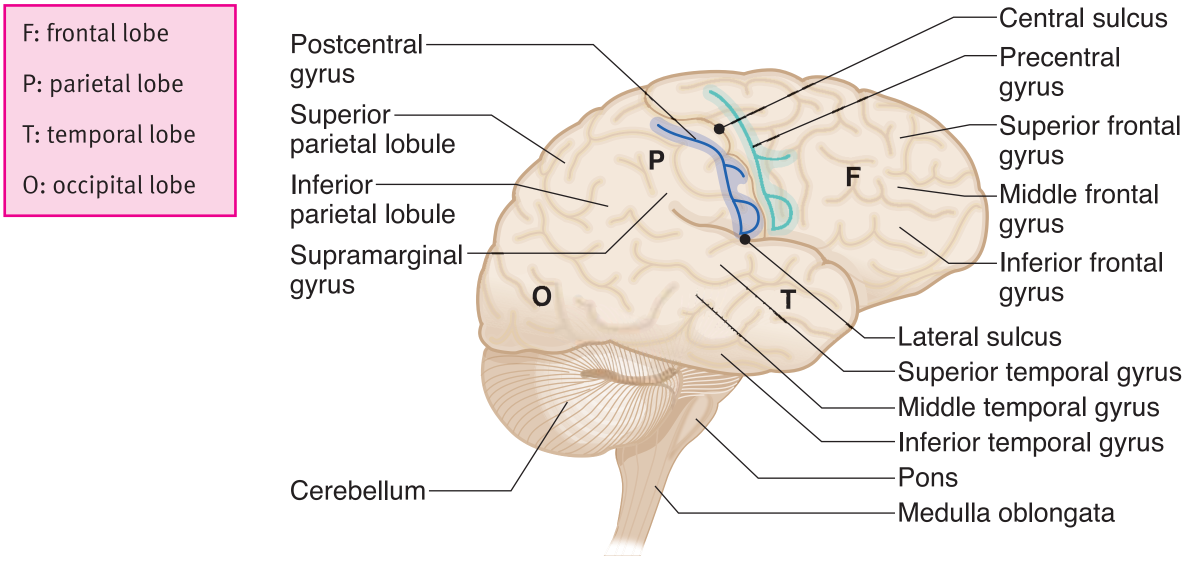


Figure III-10-1. Lateral View of the Right Cerebral Hemisphere

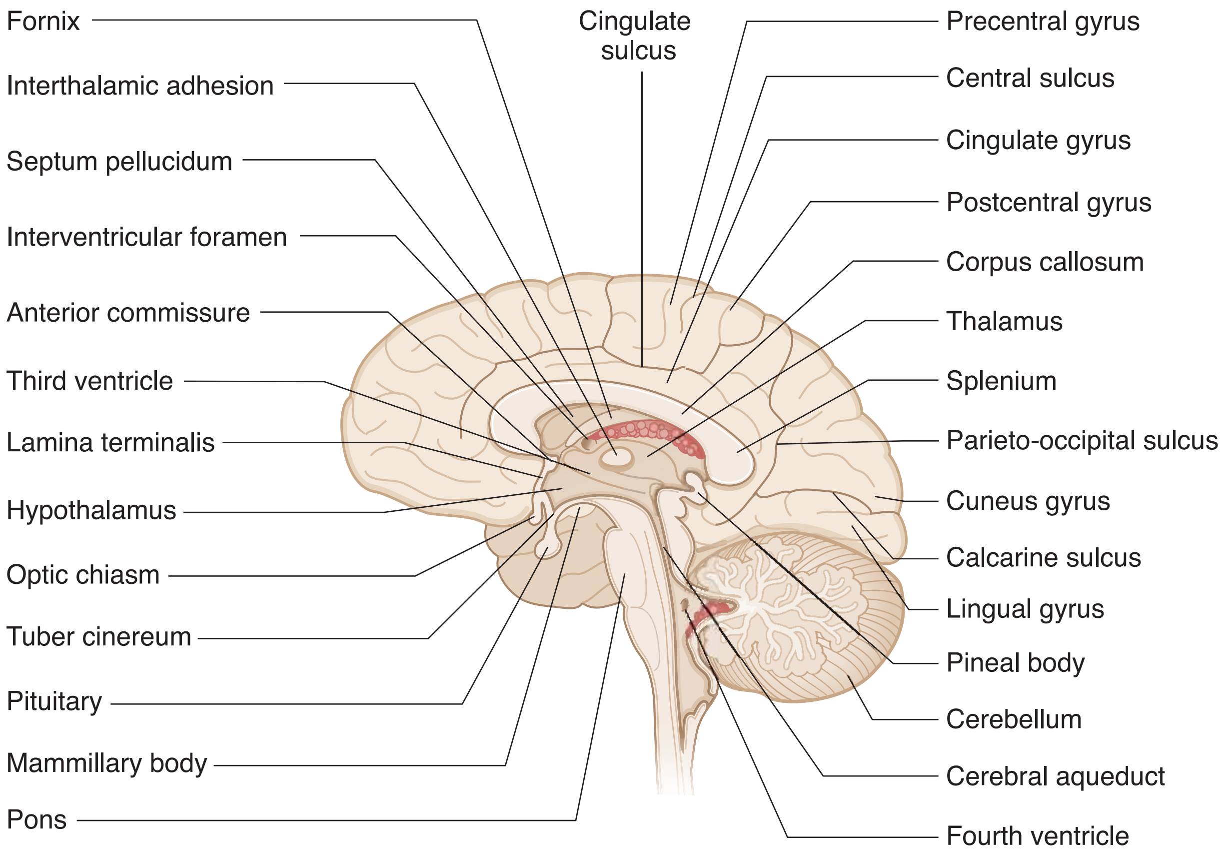


Figure III-10-2. Medial View of the Right Cerebral Hemisphere

About 90% of the cortex is composed of 6 layers, which form the neocortex (Figure III-10-5). The olfactory cortex and hippocampal formation are 3-layered structures and together comprise the allocortex. All of the neocortex contains

a 6-layer cellular arrangement, but the actual structure varies considerably between different locations. On the basis of these variations in the cytoarchitecture, Brodmann divided the cortex into 47 areas, but only a few Brodmann numbers are used synonymously with functionally specific cortical areas.

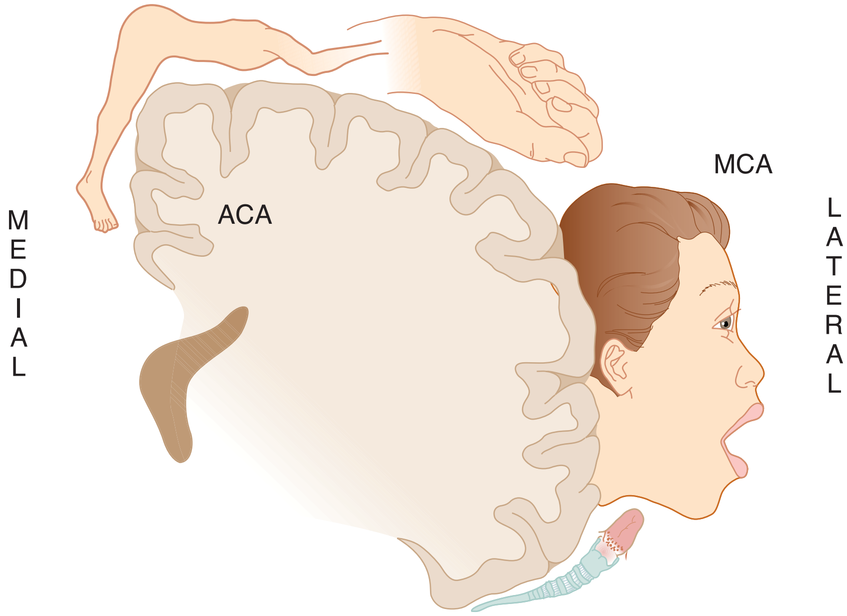


Figure III-10-3. Motor Homunculus in Precentral Gyrus (Area 4) Frontal Lobe (Coronal Section)

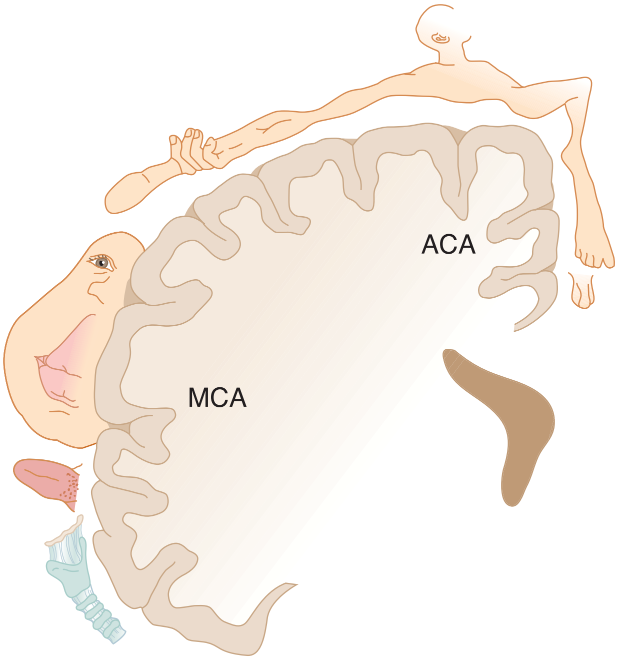


Figure III-10-4. Sensory Homunculus in Postcentral Gyrus (Areas 3, 1, 2) Parietal Lobe (Coronal Section)

Note

The internal granular layer is the site of termination of the thalamocortical projections. In primary visual cortex, these fibers form a distinct Line of Gennari. The internal pyramidal layer gives rise to axons that form the corticospinal and corticobulbar tracts.

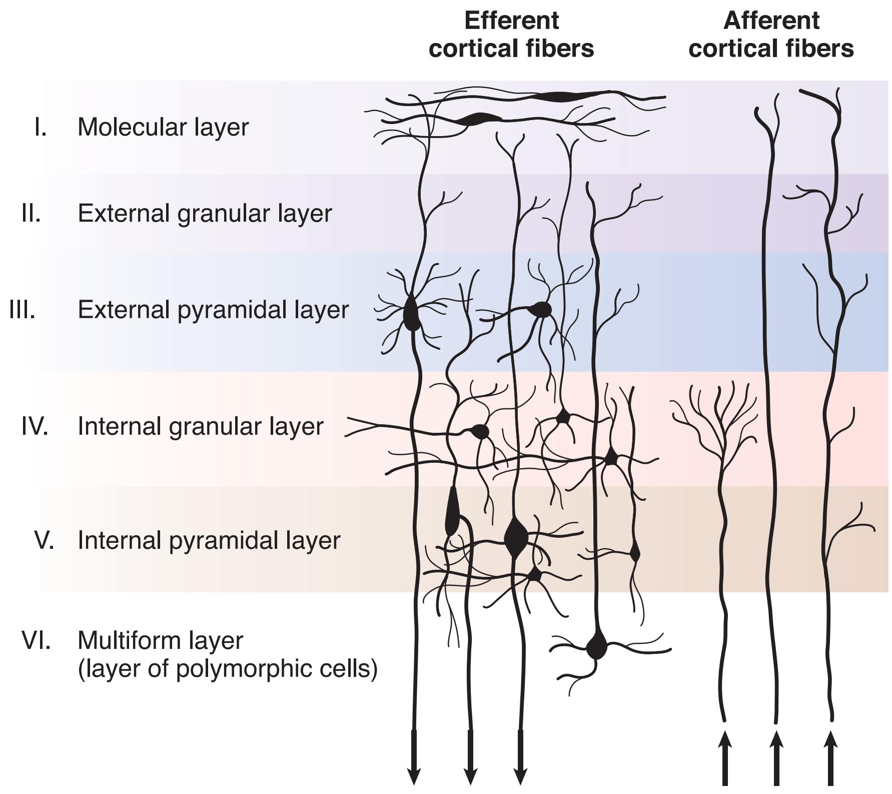


Figure III-10-5. The 6-Layered Neocortex

LANGUAGE AND THE DOMINANT HEMISPHERE

Most people (about 80%) are right-handed, which implies that the left side of the brain has more highly developed hand-controlling circuits. In the vast majority of right-handed people, speech and language functions are also predominantly organized in the left hemisphere. Most left-handed people show language functions bilaterally, although a few, with strong left-handed preferences, show right-sided speech and language functions.

BLOOD SUPPLY

The cortex is supplied by the 2 internal carotid arteries and the 2 vertebral arteries (Figures III-10-6 and III-10-7). On the base (or inferior surface) of the brain, branches of the internal carotid arteries and the basilar artery anastomose to form the circle of Willis. The anterior part of the circle lies in front of the optic chiasm, whereas the posterior part is situated just below the mammillary bodies. The circle of Willis is formed by the terminal part of the internal carotid arteries; the proximal parts of the anterior and posterior cerebral arteries and the anterior and posterior communicating arteries. The middle, anterior, and posterior cerebral arteries, which arise from the circle of Willis, supply all of the cerebral cortex, basal ganglia, and diencephalon.

The internal carotid artery arises from the bifurcation of the common carotid and enters the skull through the carotid canal. It enters the subarachnoid space and terminates by dividing into the anterior and middle cerebral arteries.

Just before splitting into the middle and anterior cerebral arteries, the internal carotid artery gives rise to the ophthalmic artery. The ophthalmic artery enters the orbit through the optic canal and supplies the eye, including the retina and optic nerve.

The middle cerebral artery is the larger terminal branch of the internal carotid artery. It supplies the bulk of the lateral surface of the hemisphere. Exceptions are the superior inch of the frontal and parietal lobes, which are supplied by the anterior cerebral artery, and the inferior part of the temporal lobe and the occipital pole, which are supplied by the posterior cerebral artery. The middle cerebral artery also supplies the genu and posterior limb of the internal capsule and the basal ganglia.

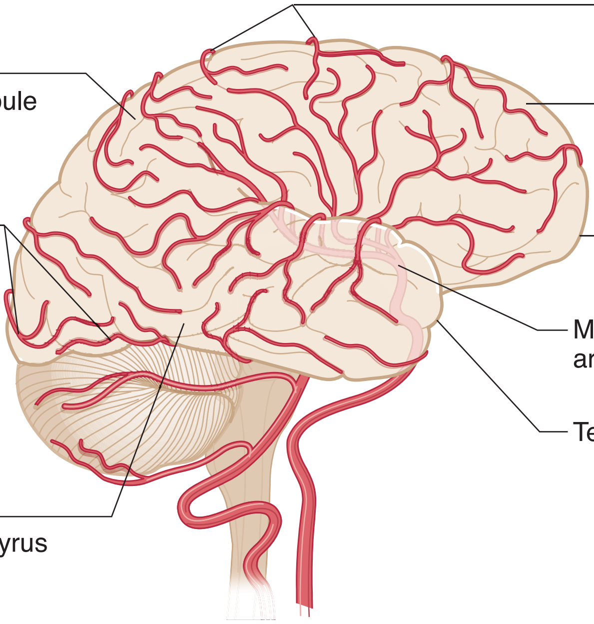


Figure III-10-6. The Distributions of the Cerebral Arteries: Part 1

The anterior cerebral artery is the smaller terminal branch of the internal carotid artery. It is connected to the opposite anterior cerebral artery by the anterior communicating artery, completing the anterior part of the circle of Willis. The anterior cerebral artery supplies the medial surface of the frontal and parietal lobes, which include motor and sensory cortical areas for the pelvis and lower limbs. The anterior cerebral artery also supplies the anterior four-fifths of the corpus callosum and approximately 1 inch of the frontal and parietal cortex on the superior aspect of the lateral aspect of the hemisphere.

Occlusion of the anterior cerebral artery results in spastic paresis of the contralateral lower limb and anesthesia of the contralateral lower limb. Urinary incontinence may be present, but this usually occurs only with bilateral damage. A transcortical apraxia of the left limbs may result from involvement of the anterior portion of the corpus callosum. A transcortical apraxia occurs because the left hemisphere (language dominant) has been disconnected from the motor cortex of the right hemisphere. The anterior cerebral artery also supplies the anterior limb of the internal capsule.

Clinical Correlate

Occlusion of the middle cerebral artery results in spastic paresis of the contralateral lower face and upper limb and anesthesia of the contralateral face and upper limb.

An aphasia (e.g., Broca, Wernicke, or conduction) may result when branches of the left middle cerebral artery are affected, and left-sided neglect may be seen with a blockage of branches of the right middle cerebral artery to the right parietal lobe.

The middle cerebral artery also supplies the proximal parts of the visual radiations as they emerge from the lateral geniculate nucleus of the thalamus and course in Meyer's loop. These fibers course into the temporal lobe before looping posteriorly to rejoin the rest of the visual radiation fibers.

Occlusion of the branches that supply Meyer's loop fibers in the temporal lobe results in a contralateral superior quadrantanopsia.

Note

The middle cerebral artery (MCA) supplies:

- the lateral surface of the frontal, parietal, and upper temporal lobes
- the posterior limb and genu of the internal capsule
- most of the basal ganglia

Note

The anterior cerebral artery (ACA) supplies medial surface of frontal and parietal lobes; anterior 4/5 of corpus callosum; anterior limb of internal capsule

The posterior cerebral artery (PCA) supplies occipital lobe; lower temporal lobe; splenium; midbrain

Clinical Correlate

The most common aneurysm site in the circle of Willis is where the anterior communicating artery joins an anterior cerebral artery.

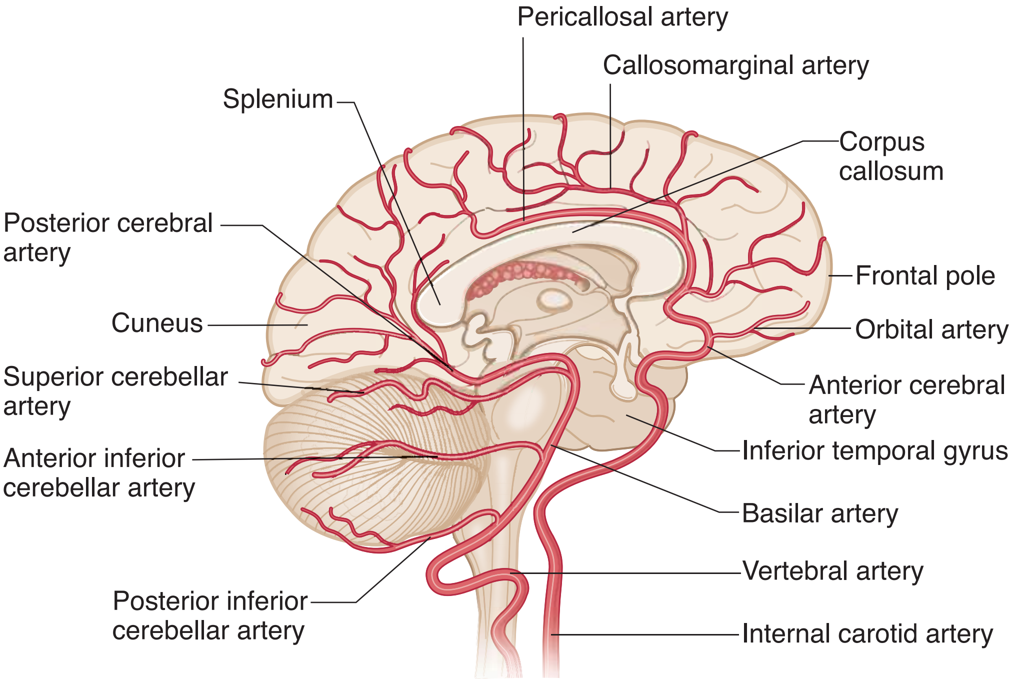


Figure III-10-7. The Distributions of the Cerebral Arteries: Part 2

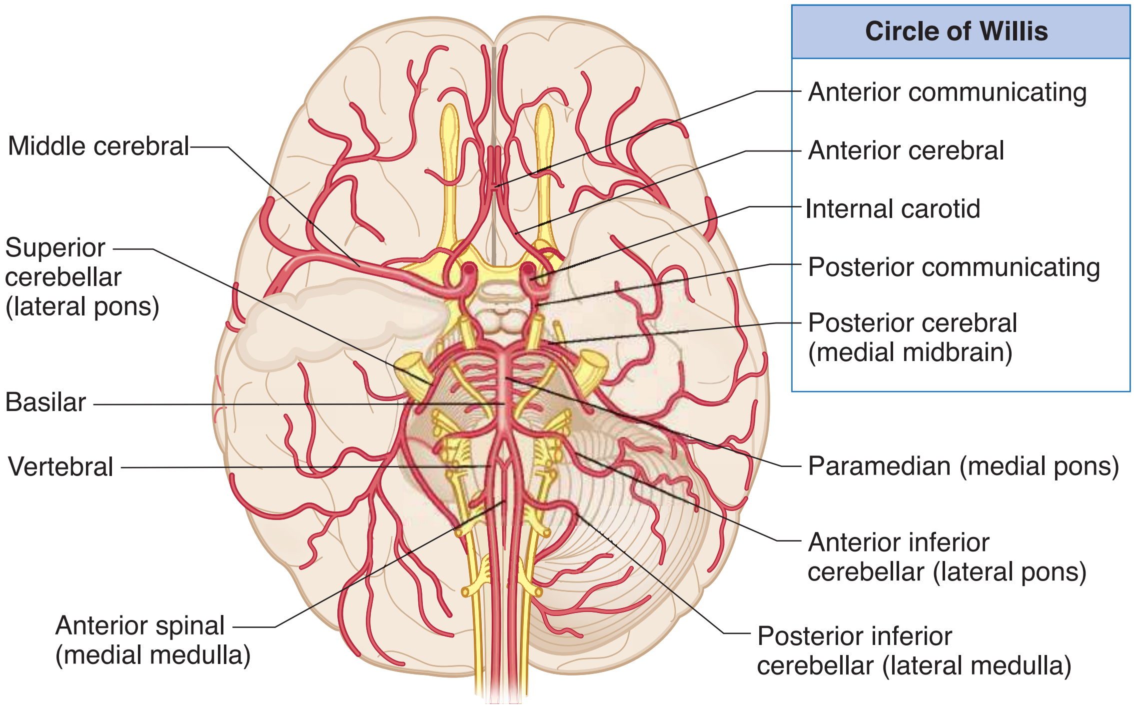


Figure III-10-8. Arterial Supply of the Brain

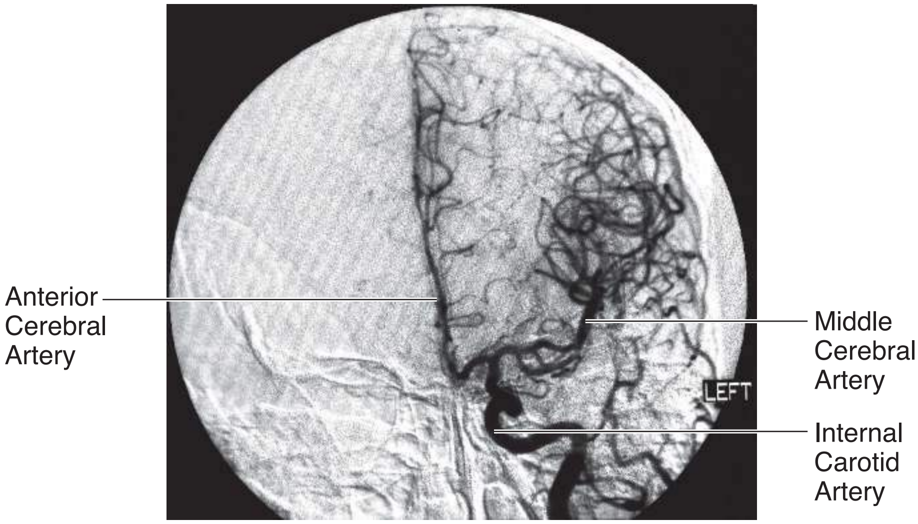


Figure III-10-9. Anteroposterior View of Left Internal Carotid

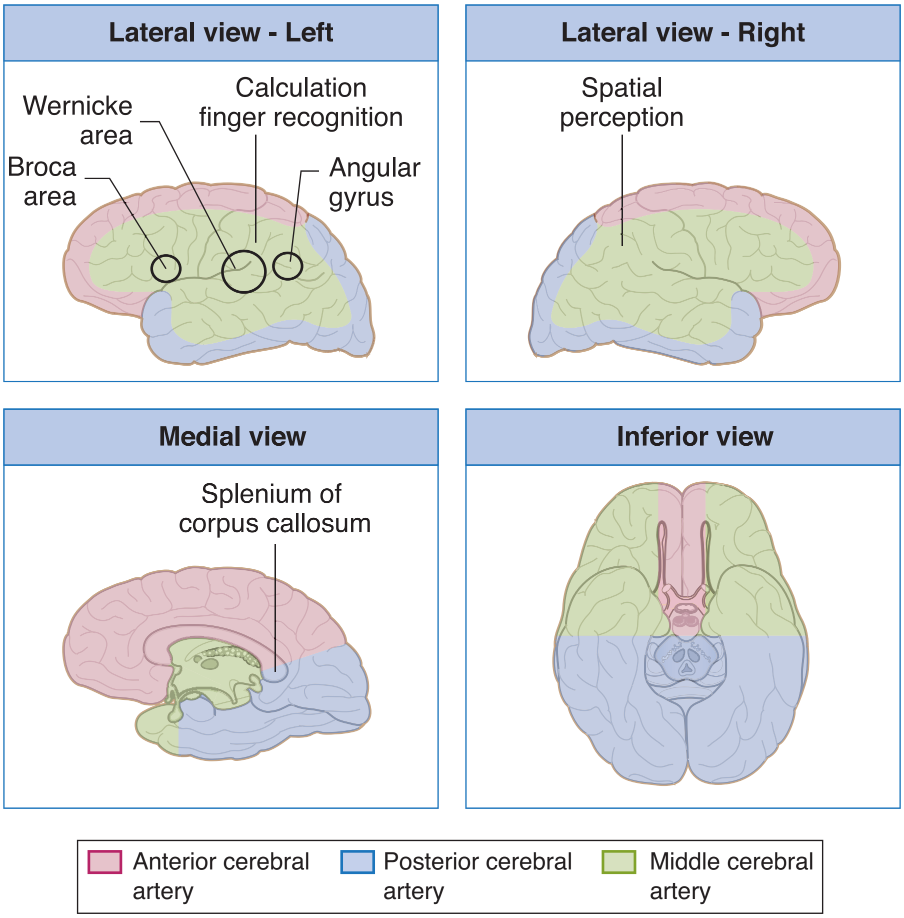


Figure III-10-10. Territories Supplied by the Cerebral Arteries

The posterior cerebral artery is formed by the terminal bifurcation of the basilar artery. The posterior communicating artery arises near the termination of the internal carotid artery and passes posteriorly to join the posterior cerebral artery. The posterior communicating arteries complete the circle of Willis by joining the vertebrobasilar and carotid circulations. The posterior cerebral artery supplies the occipital and temporal cortex on the inferior and lateral surfaces of the hemisphere, the occipital lobe and posterior 2/3 of the temporal lobe on the medial surface of the hemisphere, and the thalamus and subthalamic nucleus.

Occlusion of the posterior cerebral artery results in a homonymous hemianopia of the contralateral visual field with macular sparing.

Table III-10-1. Cerebrovascular Disorders

Disorder	Types	Key Concepts
Cerebral infarcts	Thrombotic	**Anemic/pale** infarct; usually atherosclerotic complication
	Embolic	**Hemorrhagic/red** infarct; from heart or atherosclerotic plaques; **middle cerebral artery** most vulnerable to emboli
	Hypotension	"**Watershed**" areas and deep cortical layers most affected
	Hypertension	Lacunar infarcts; basal ganglia, internal capsule, and pons most affected
Hemorrhages	Epidural hematoma	Almost always traumatic Rupture of **middle meningeal artery** after skull fracture Lucid interval before loss of consciousness ("talk and die" syndrome)
	Subdural hematoma	Usually caused by trauma Rupture of **bridging veins** (drain brain to dural sinuses)
	Subarachnoid hemorrhage	**Ruptured berry aneurysm** is most frequent cause Predisposing factors: Marfan syndrome, Ehlers-Danlos type 4, adult polycystic kidney disease, hypertension, smoking
	Intracerebral hemorrhage	Common causes: hypertension, trauma, infarction

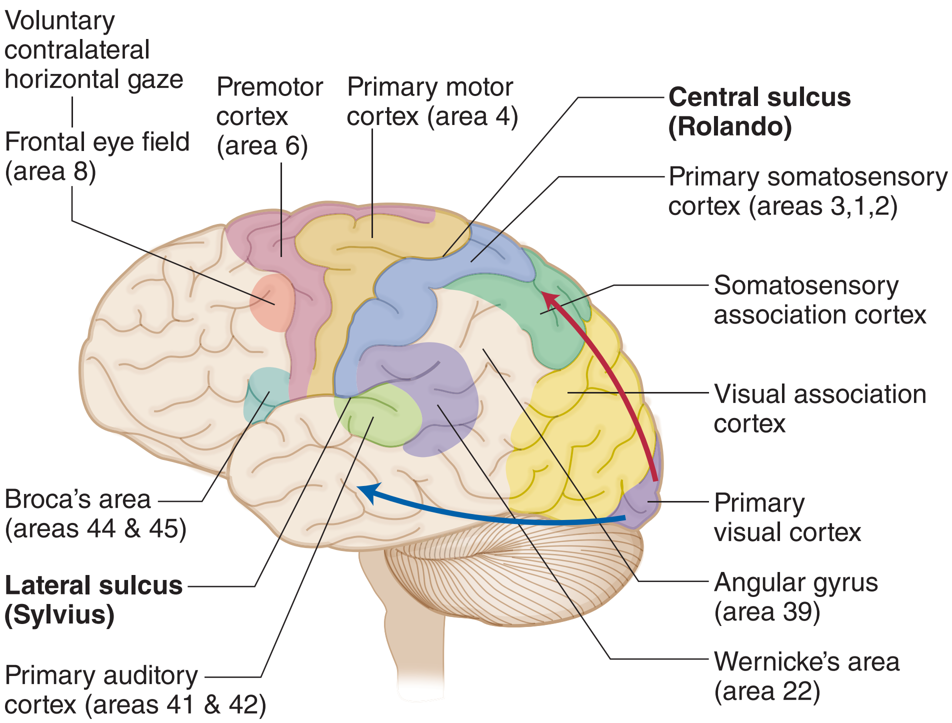


Figure III-10-11. Cerebral Cortex: Functional Areas of Left (Dominant) Hemisphere

Frontal Lobe

A large part of the frontal cortex rostral to the central sulcus is related to the control of movements, primarily on the opposite side of the body. These areas include primary motor cortex (Brodmann area 4), premotor cortex (area 6), the frontal eye field (area 8), and the motor speech areas of Broca (area 44 and 45). Traditionally, area 4 is considered the primary motor cortex. It is in the precentral gyrus, immediately anterior to the central sulcus, and contains an orderly skeletal motor map of the contralateral side of the body.

The muscles of the head are represented most ventrally closest to the lateral fissure; then, proceeding dorsally, are the regions for the neck, upper limb, and trunk on the lateral aspect of the hemisphere. On the medial aspect of the hemisphere is the motor representation for the pelvis and lower limb.

Premotor cortex

Just anterior to area 4 is the premotor cortex (area 6). Neurons here are particularly active prior to the activation of area 4 neurons, so it is thought that the premotor cortex is involved in the planning of motor activities. Damage here results in an apraxia, a disruption of the patterning and execution of learned motor movements. Individual movements are intact, and there is no weakness, but the patient is unable to perform movements in the correct sequence.

Clinical Correlate

Lesion of the Frontal Eye Field

The frontal eye field lies in front of the motor cortex in Brodmann area 8. This cortical area is the center for contralateral horizontal gaze. A lesion here results in an inability to make voluntary eye movements toward the contralateral side. Because the activity of the intact frontal eye field in the opposite cortex would also be unopposed after such a lesion, the result is conjugate slow deviation of the eyes toward the side of the lesion.

If motor cortex is involved in the lesion, the patient may have a contralateral spastic paresis. The intact frontal eye field in the opposite hemisphere deviates the eyes **away** from the paralyzed limbs.

Prefrontal cortex

The prefrontal cortex is located in front of the premotor area and represents about a quarter of the entire cerebral cortex in the human brain. This area is involved in organizing and planning the intellectual and emotional aspects of behavior, much as the adjacent premotor cortex is involved in planning its motor aspects.

Clinical Correlate

Lesions in the Prefrontal Area

Lesions in the prefrontal area produce what is called the frontal lobe syndrome. The patient cannot concentrate and is easily distracted; there is a general lack of initiative, foresight, and perspective. Another common aspect is apathy (i.e., severe emotional indifference). Apathy is usually associated with abulia, a slowing of intellectual faculties, slow speech, and decreased participation in social interactions. Prefrontal lesions also result in the emergence of infantile suckling or grasp reflexes that are suppressed in adults. In the suckling reflex, touching the cheek causes the head to turn toward the side of the stimulus as the mouth searches for a nipple to suckle. In the grasp reflex, touching the palm of the hand results in a reflex closing of the fingers, which allows an infant to grasp anything that touches the hand.

Clinical Correlate

Expressive Aphasia

Broca area is just anterior to the motor cortex region that provides upper motoneuron innervation of cranial nerve motor nuclei. This area in the left or dominant hemisphere is the center for motor speech and corresponds to Brodmann areas 44 and 45. Damage to Broca area produces a motor, nonfluent, or expressive aphasia that reflects a difficulty in piecing together words to produce expressive speech. Patients with this lesion can understand written and spoken language but normally say almost nothing. When pressed on a question such as “What did you do today?” they might reply “Went town.” The ability to write is usually also affected in a similar way (agraphia) in all aphasias, although the hand used for writing can be used normally in all other tasks. Patients are keenly aware of and frustrated by an expressive aphasia, because of their lack of the ability to verbalize their thoughts orally or in writing.

Broca area damage often extends posteriorly into the primary motor cortex and might be combined with a contralateral paralysis of the muscles of the lower face, resulting in a drooping of the corner of the mouth. If the lesion is larger, the patient might have a spastic hemiparesis of the contralateral upper limb.

Parietal Lobe

Primary somatosensory cortex

The parietal lobe begins just posterior to the central sulcus with the postcentral gyrus. The postcentral gyrus corresponds to Brodmann areas 3, 1, and 2 and contains primary somatosensory cortex. Like primary motor cortex, there is a similar somatotopic representation of the body here, with head, neck, upper limb, and trunk represented on the lateral aspect of the hemisphere, and pelvis and lower limb represented medially. These areas are concerned with discriminative touch, vibration, position sense, pain, and temperature. Lesions in somatosensory cortex result in impairment of all somatic sensations on the opposite side of the body, including the face and scalp.

Posterior parietal association cortex

Just posterior and ventral to the somatosensory areas is the posterior parietal association cortex, including Brodmann areas 5 and 7.

Clinical Correlate

Lesions, usually in the dominant hemisphere and which include areas 5 and 7 of the posterior parietal association areas, often result in apraxia (also seen with lesions to the premotor cortex). Apraxia is a disruption of the patterning and execution of learned motor movements. This deficit seems to reflect a lack of understanding how to organize the performance of a pattern of movements (i.e., what should be done first, then next, etc.). The patient may be unable, for example, to draw a simple diagram (constructional apraxia) or describe how to get from his home to his work.

Another deficit, with lesions of areas 5 and 7 is astereognosia (inability to recognize objects by touch). There is no loss of tactile or proprioceptive sensation; rather, it is the integration of visual and somatosensory information that is impaired. Both apraxia and astereognosia are more common after left hemisphere damage than in right hemisphere damage. The astereognosia is usually confined to the contralateral side of the body; in contrast, apraxia is usually bilateral. Apraxia is probably a result of the loss of input to the premotor cortex (area 6), which is involved in the actual organization of motor movements into a goal-directed pattern.

Wernicke area

The inferior part of the parietal lobe and adjacent part of the temporal lobe in the dominant (left) hemisphere, known as Wernicke area, are cortical regions that function in language comprehension. At a minimum, Wernicke area consists of area 22 in the temporal lobe but may also include areas 39 and 40 in the parietal lobe. Areas 39 (the angular gyrus) and 40 (the supramarginal gyrus) are regions of convergence of visual, auditory, and somatosensory information.

Note

Any blockage of the left middle cerebral artery that results in an aphasia (Broca Wernicke, conduction) or Gerstmann syndrome will also result in **agraphia.**

Clinical Correlate

Receptive Aphasia

Lesions in area 22 in the temporal lobe and area 39 or 40 in the parietal lobe produce a fluent, receptive, or Wernicke aphasia. The patient with Wernicke aphasia cannot comprehend spoken language and may or may not be able to read (alexia) depending on the extent of the lesion. The deficit is characterized by fluent verbalization but lacks meaning. Patients are paraphasic, often misusing words as if speaking using a "word salad."

Patients with Wernicke aphasia are generally unaware of their deficit and show no distress as a result of their condition.

Gerstmann Syndrome

If the lesion is confined to just the angular gyrus (area 39), the result is a loss of ability to comprehend written language (alexia) and to write it (agraphia), but spoken language may be understood. Alexia with agraphia in pure angular gyrus lesions is often seen with 3 other unique symptoms: acalculia (loss of the ability to perform simple arithmetic problems), finger agnosia (inability to recognize one's fingers), and right–left disorientation. This constellation of deficits constitutes Gerstmann syndrome and underscores the role of this cortical area in the integration of how children begin to count, add, and subtract using their fingers.

Conduction Aphasia

There is a large fiber bundle connecting areas 22, 39, and 40 with Broca area in the frontal lobe, known as the superior longitudinal fasciculus (or the arcuate fasciculus). A lesion affecting this fiber bundle results in a conduction aphasia. In this patient, verbal output is fluent, but there are many paraphrases and word-finding pauses. Both verbal and visual language comprehension are also normal, but if asked to, the patient cannot repeat words or execute verbal commands by an examiner (such as "Count backwards beginning at 100") and also demonstrates poor object naming. This is an example of a disconnect syndrome in which the deficit represents an inability to send information from one cortical area to another. As with an expressive aphasia, these patients are aware of the deficit and are frustrated by their inability to execute a verbal command that they fully understand.

Transcortical Apraxia

Lesions to the corpus callosum caused by an infarct of the anterior cerebral artery may result in another type of disconnect syndrome known as a transcortical apraxia. As in other cases of apraxia, there is no motor weakness, but the patient cannot execute a command to move the left arm. They understand the command, which is perceived in the Wernicke area of the left hemisphere, but the callosal lesion disconnects the Wernicke area from the right primary motor cortex so that the command cannot be executed. The patient is still able to execute a command to move the right arm because Wernicke area in the left hemisphere is able to communicate with the left primary motor cortex without using the corpus callosum.

Asomatognosia

The integration of visual and somatosensory information is important for the formation of the "body image" and awareness of the body and its position in space. Widespread lesions in areas 7, 39, and 40 in the nondominant right parietal lobe may result in unawareness or neglect of the contralateral half of the body, known as asomatognosia. Although somatic sensation is intact, the patients ignore half of their body and may fail to dress, undress, or wash the affected (left) side. Patients will have no visual field deficits, so they can see, but deny the existence of things in the left visual field. Asking them to bisect a horizontal line produces a point well to the right of true center. If asked to draw a clock face from memory, they will draw only the numbers on the right side, ignoring those on the left. Patients may deny that the left arm or leg belongs to them when the affected limb is passively brought into their field of vision. Patients may also deny their deficit, an anosognosia.

Occipital Lobe

The occipital lobe is essential for the reception and recognition of visual stimuli and contains primary visual and visual association cortex.

Visual cortex

The visual cortex is divided into striate (area 17) and extrastriate (areas 18 and 19). Area 17, also referred to as the primary visual cortex, lies on the medial portion of the occipital lobe on either side of the calcarine sulcus. Its major thalamic input is from the lateral geniculate nucleus. Some input fibers are gathered in a thick bundle that can be visible on the cut surface of the gross brain, called the line of Gennari. The retinal surface (and therefore the visual field) is represented in an orderly manner on the surface of area 17, such that damage to a discrete part of area 17 will produce a scotoma (i.e., a blind spot) in the corresponding portion of the visual field. A unilateral lesion inside area 17 results in a contralateral homonymous hemianopsia with macular sparing, usually caused by an infarct of a branch of the posterior cerebral artery. The area of the macula of the retina containing the fovea is spared because of a dual blood supply from both the posterior and middle cerebral arteries. The actual cortical area serving the macula is represented in the most posterior part of the occipital lobe. Blows to the back of the head or a blockage in occipital branches of the middle cerebral artery that supply this area may produce loss of macular representation of the visual fields. Bilateral visual cortex lesions result in cortical blindness; the patient cannot see, but pupillary reflexes are intact.

Visual association cortex

Anterior to the primary visual or striate cortex are extensive areas of visual association cortex. Visual association cortex is distributed throughout the entire occipital lobe and in the posterior parts of the parietal and temporal lobes. These regions receive fibers from the striate cortex and integrate complex visual input from both hemispheres. From the retina to the visual association cortex, information about form and color, versus motion, depth and spatial information are processed separately. Form and color information is processed by the parvocellular-blob system. This "cone stream" originates mainly in the central part of the retina, relays through separate layers of the lateral geniculate, and projects to blob zones of primary visual cortex.

Blob zones project to the inferior part of the temporal lobe in areas 20 and 21. Unilateral lesions here result in achromatopsia, a complete loss of color vision in the contralateral hemifields. Patients see everything in shades of gray. Additionally, these patients may also present with prosopagnosia, an inability to recognize faces.

Motion and depth are processed by the magnocellular system. This "rod stream" originates in the peripheral part of the retina, relays through separate layers of the lateral geniculate, and projects to thick stripe zones of primary visual cortex. Striped areas project through the middle temporal lobe to the parietal lobe in areas 18 and 19. Lesions here result in a deficit in perceiving visual motion; visual fields, color vision, and reading are unaffected.

Clinical Correlate

Visual Agnosia

Damage to parts of the temporal lobes involving the cone stream produces a visual agnosia. Visual agnosia is the inability to recognize visual patterns (including objects) in the absence of a visual field deficit. For example, you might show a patient with an object agnosia a pair of glasses, and the patient would describe them as 2 circles and a bar. Lesions in areas 20 and 21 of the temporal lobe that also include some destruction of adjacent occipital lobe in either hemisphere result in prosopagnosia, a specific inability to recognize faces. The patient can usually read and name objects. The deficiency is an inability to form associations between faces and identities. On hearing the voice of the same person, the patient can immediately identify the person.

Alexia Without Agraphia

A principal "higher-order" deficit associated with occipital lobe damage is alexia without agraphia (or pure word blindness). The patients are unable to read at all and, curiously, often have a color anomia (inability to name colors). However, they are able to write. This is another example of a disconnect syndrome in which information from the occipital lobe is not available to the parietal or frontal lobes to either understand or express what has been seen.

(Recall that alexia *with* agraphia—inability to read or write—occurs with lesions encompassing the angular gyrus in the dominant parietal lobe.) The cause of the syndrome is usually an infarction of the left posterior cerebral artery that affects not only the anterior part of the occipital lobe but the splenium of the corpus callosum. Involvement of the left occipital cortex results in a right homonymous hemianopsia with macular sparing. Involvement of the splenium of the corpus callosum prevents visual information from the intact right occipital cortex from reaching language comprehension centers in the left hemisphere. Patients can see words in the left visual field but do not understand what the words mean.

Temporal Lobe

Primary auditory cortex

On its superior and lateral aspect, the temporal lobe contains the primary auditory cortex. Auditory cortex (areas 41 and 42) is located on the 2 transverse gyri of Heschl, which cross the superior temporal lobe deep within the lateral sulcus. Much of the remaining superior temporal gyrus is occupied by area 22 (auditory association cortex), which receives a considerable projection from both areas 41 and 42 and projects widely to both parietal and occipital cortices.

Patients with unilateral damage to the primary auditory cortex show little loss of auditory sensitivity but have some difficulty in localizing sounds in the contralateral sound field. Area 22 is a component of Wernicke area in the dominant hemisphere, and lesions here produce a Wernicke aphasia.

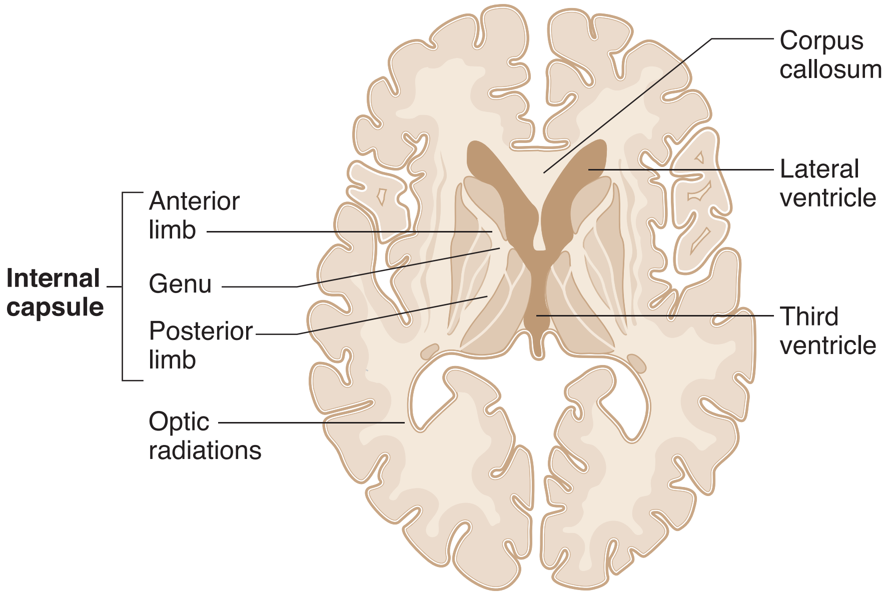


Figure III-10-12. Internal Capsule: Arterial Supply

Table III-10-2. Internal Capsule: Arterial Supply

Internal Capsule	Arterial Supply	Tracts
Anterior limb	Medial striate br. of ACA	Thalamocortical
Genu	Lenticulostriate br. of MCA	Corticobulbar
Posterior limb	Lenticulostriate br. of MCA	Corticospinal, all somatosensory thalamocortical projections

Note: The posterior cerebral artery also supplies the optic radiations.

Abbreviations: ACA, anterior cerebral artery; MCA, middle cerebral artery

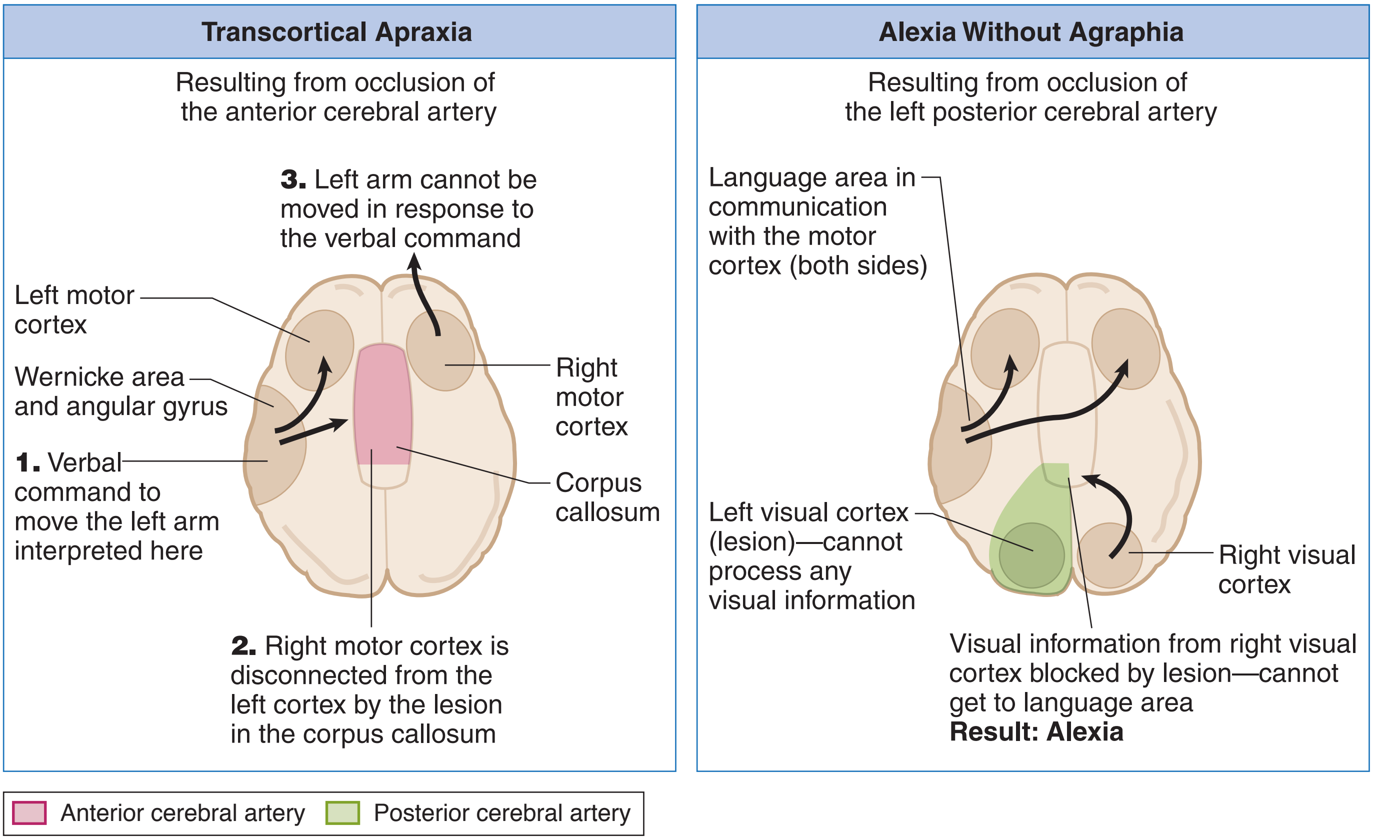


Figure III-10-13. Disconnect Syndromes

Table III-10-3. CNS Blood Supply and Stroke-related Deficits

System	Primary Arteries	Branches	Supplies	Deficits after Stroke
Vertebrobasilar (posterior circulation)	**Vertebral arteries**	**Anterior spinal artery**	Anterior two-thirds of spinal cord	Dorsal columns spared; all else bilateral
		Posterior inferior cerebellar (PICA)	Dorsolateral medulla	See Brain-Stem Lesions in Chapter IV-5.
	Basilar artery	Pontine arteries	Base of pons	
		Anterior inferior cerebellar artery (AICA)	Inferior cerebellum, cerebellar nuclei	
		Superior cerebellar artery	Dorsal cerebellar hemispheres; superior cerebellar peduncle	
		Labyrinthine artery (sometimes arises from AICA)	Inner ear	
	Posterior cerebral arteries	—	Midbrain, thalamus, occipital lobe	**Contralateral hemianopia with macular sparing** Alexia without agraphia*
Internal carotid (anterior circulation)	Ophthalmic artery	Central artery of retina	Retina	Blindness
	Posterior communicating artery	—	—	Second most common **aneurysm** site (often with CN III palsy)
	Anterior cerebral artery	—	Primary motor and sensory cortex (leg/foot)	Contralateral spastic paralysis and anesthesia of **lower limb** Frontal lobe abnormalities
	Anterior communicating artery	—	—	Most common site of **aneurysm**
	Middle cerebral artery	Outer cortical	Lateral convexity of hemispheres	Contralateral spastic paralysis and anesthesia of **upper limb/face** **Gaze palsy Aphasia*** Gerstmann syndrome* Hemi inattention and neglect of contralateral body†
		Lenticulostriate	Internal capsule, caudate, putamen, globus pallidus	

*If dominant hemisphere is affected (usually the left)

†Right parietal lobe lesion

Table III-10-4. Key Features of Lobes

Lobes	Important Regions	Deficit After Lesion
Frontal	Primary motor and premotor cortex	Contralateral spastic paresis (region depends on area of homunculus affected), premotor: apraxia
	Frontal eye fields	Eyes deviate to ipsilateral side
	Broca speech area* (Areas 44, 45)	**Broca aphasia (expressive, nonfluent aphasia):** patient can understand written and spoken language, but speech and writing are slow and effortful; patients are aware of their problem; often associated with right arm weakness and right lower face weakness.
	Prefrontal cortex	**Frontal lobe syndrome:** symptoms can include poor judgment, difficulty concentrating and problem solving, apathy, inappropriate social behavior
Parietal	Primary somatosensory cortex	Contralateral hemihypesthesia (region depends on area of homunculus affected)
	Superior parietal lobule	Contralateral astereognosis/apraxia
	Inferior parietal lobule (Angular gyrus; Area 39)	**Gerstmann syndrome** (if dominant hemisphere): right/left confusion, alexia, dyscalculia and dysgraphia, finger agnosia, contralateral hemianopia or lower quadrantanopia; unilateral neglect (nondominant)
Temporal	Primary auditory cortex	Bilateral damage → deafness Unilateral leads to slight hearing loss
	Wernicke area* (Area 22)	**Wernicke aphasia (receptive, fluent aphasia):** patient cannot understand any form of language; speech is fast and fluent, but not comprehensible
	Hippocampus	Bilateral lesions lead to inability to consolidate short-term to long-term memory
	Amygdala	**Klüver-Bucy syndrome:** hyperphagia, hypersexuality, visual agnosia
	Olfactory bulb, tract, primary cortex	Ipsilateral anosmia
	Meyer loop (visual radiations)	Contralateral upper quadrantanopia (“pie in the sky”)
Occipital	Primary visual cortex	Cortical blindness if bilateral; macular sparing hemianopia

*In the dominant hemisphere. Eighty percent of people are left-hemisphere dominant.

Limbic System 11

Learning Objectives

- ❑ Solve problems concerning general features
- ❑ Solve problems concerning olfactory system
- ❑ Demonstrate understanding of limbic system

GENERAL FEATURES

The limbic system is involved in emotion, memory, attention, feeding, and mating behaviors. It consists of a core of cortical and diencephalic structures found on the medial aspect of the hemisphere. A prominent structure in the limbic system is the hippocampal formation on the medial aspect of the temporal lobe. The hippocampal formation extends along the floor of the inferior horn of the lateral ventricle in the temporal lobe and includes the hippocampus, the dentate gyrus, the subiculum, and adjacent entorhinal cortex. The hippocampus is characterized by a 3-layered cerebral cortex. Other limbic-related structures include the amygdala, which is located deep in the medial part of the anterior temporal lobe rostral to the hippocampus, and the septal nuclei, located medially between the anterior horns of the lateral ventricle. The limbic system is interconnected with thalamic and hypothalamic structures, including the anterior and dorsomedial nuclei of the thalamus and the mammillary bodies of the hypothalamus. The cingulate gyrus is the main limbic cortical area. The cingulate gyrus is located on the medial surface of each hemisphere above the corpus callosum. Limbic-related structures also project to wide areas of the prefrontal cortex.

Note

Functions of the Limbic System

- Visceral—smell
- Sex drive
- Memory/Learning
- Behavior and emotions

OLFACTORY SYSTEM

Central projections of olfactory structures reach parts of the temporal lobe without a thalamic relay and the amygdala. The olfactory nerve consists of numerous fascicles of the central processes of bipolar neurons, which reach the anterior cranial fossa from the nasal cavity through openings in the cribriform plate of the ethmoid bone. These primary olfactory neurons differ from other primary sensory neurons in 2 ways. First, the cell bodies of these neurons, which lie scattered in the olfactory mucosa, are not collected together in a sensory ganglion, and second, primary olfactory neurons are continuously replaced. The life span of these cells ranges from 30 to 120 days in mammals.

Within the mucosa of the nasal cavity, the peripheral process of the primary olfactory neuron ramifies to reach the surface of the mucous membrane. The central processes of primary olfactory neurons terminate by synapsing with neurons found in the olfactory bulb. The bulb is a 6-layered outgrowth of the brain that rests on the cribriform plate. Olfactory information entering the

olfactory bulb undergoes a great deal of convergence before the olfactory tract carries axons from the bulb to parts of the temporal lobe and amygdala.

Clinical Correlate

Alzheimer disease results from neurons, beginning in the hippocampus, that exhibit neurofibrillary tangles and amyloid plaques. Other nuclei affected are the cholinergic neurons in the nucleus basalis of Meynert, noradrenergic neurons in the locus coeruleus, and serotonergic neurons in the raphe nuclei. Patients with Down syndrome commonly present with Alzheimer in middle age because chromosome 21 is one site of a defective gene.

Clinical Correlate

Olfactory deficits may be incomplete (hyposmia), distorted (dysosmia), or complete (anosmia). Olfactory deficits are caused by transport problems or by damage to the primary olfactory neurons or to neurons in the olfactory pathway to the central nervous system (CNS). Head injuries that fracture the cribriform plate can tear the central processes of olfactory nerve fibers as they pass through the plate to terminate in the olfactory bulb, or they may injure the bulb itself. Because the olfactory bulb is an outgrowth of the CNS covered by meninges, separation of the bulb from the plate may tear the meninges, resulting in cerebrospinal fluid (CSF) leaking through the cribriform plate into the nasal cavity.

LIMBIC SYSTEM

The limbic system is involved in emotion, memory, attention, feeding, and mating behaviors. It consists of a core of cortical and diencephalic structures found on the medial aspect of the hemisphere. The limbic system modulates feelings, such as fear, anxiety, sadness, happiness, sexual pleasure, and familiarity.

The Papez Circuit

A summary of the simplified connections of the limbic system is expressed by the Papez circuit (Figure III-11-1). The Papez circuit oversimplifies the role of the limbic system in modulating feelings, such as fear, anxiety, sadness, happiness, sexual pleasure, and familiarity; yet, it provides a useful starting point for understanding the system. Arbitrarily, the Papez circuit begins and ends in the hippocampus. Axons of hippocampal pyramidal cells converge to form the fimbria and, finally, the fornix. The fornix projects mainly to the mammillary bodies in the hypothalamus. The mammillary bodies, in turn, project to the anterior nucleus of the thalamus by way of the mammillothalamic tract. The anterior nuclei project to the cingulate gyrus through the anterior limb of the internal capsule, and the cingulate gyrus communicates with the hippocampus through the cingulum and entorhinal cortex.

The amygdala functions to attach an emotional significance to a stimulus and helps imprint the emotional response in memory.

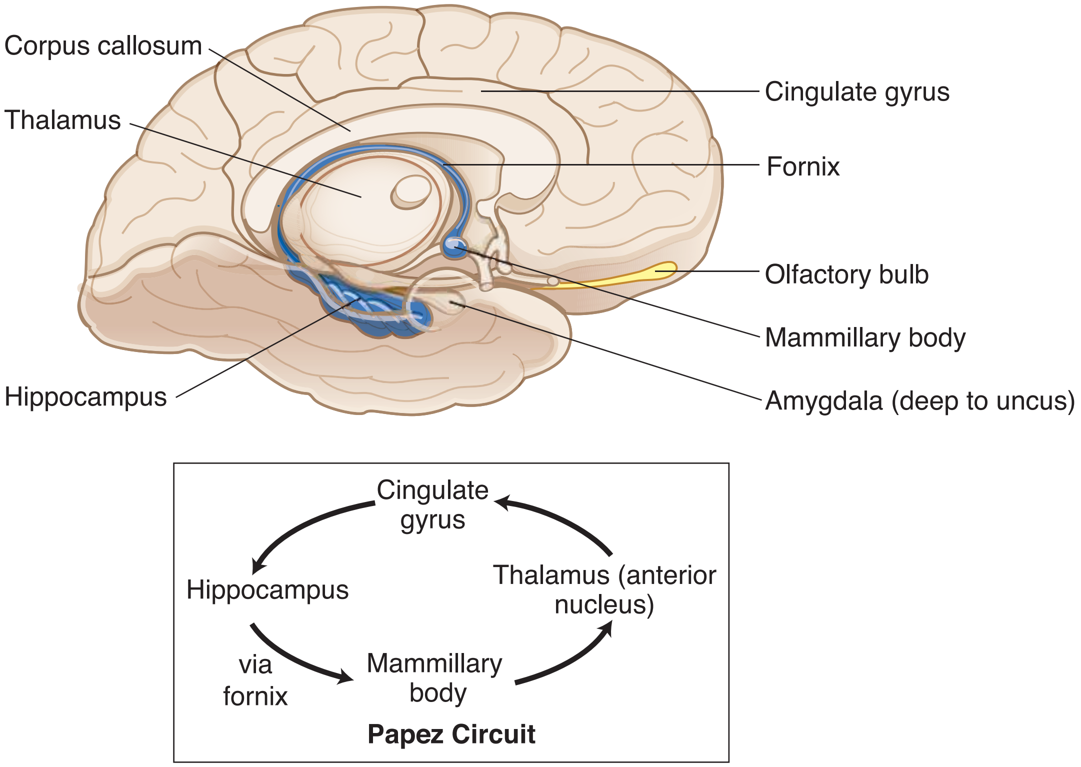


Figure III-11-1. The Limbic System and Papez Circuit

Limbic Structures and Function

- Hippocampal formation (hippocampus, dentate gyrus, the subiculum, and entorhinal cortex)
- Amygdala
- Septal nuclei
- The hippocampus is important in learning and memory. The amygdala attaches an emotional significance to a stimulus and helps imprint the emotional response in memory.

Limbic Connections

- The limbic system is interconnected with anterior and dorsomedial nuclei of the thalamus and the mammillary bodies.
- The cingulate gyrus is the main limbic cortical area.
- Limbic-related structures also project to wide areas of the prefrontal cortex.
- Central projections of olfactory structures reach parts of the temporal lobe and the amygdala.

Papez Circuit

Axons of hippocampal pyramidal cells converge to form the fimbria and, finally, the fornix. The fornix projects mainly to the mammillary bodies in

the hypothalamus. The mammillary bodies project to the anterior nucleus of the thalamus (mammillothalamic tract). The anterior nuclei project to the cingulate gyrus, and the cingulate gyrus projects to the entorhinal cortex (via the cingulum). The entorhinal cortex projects to the hippocampus (via the perforant pathway).

Clinical Correlate

Anterograde Amnesia

Bilateral damage to the medial temporal lobes including the hippocampus results in a profound loss of the ability to acquire new information, known as anterograde amnesia.

Korsakoff Syndrome

Anterograde amnesia is also observed in patients with Korsakoff syndrome. Korsakoff syndrome is seen mainly in alcoholics who have a thiamine deficiency and often follows an acute presentation of Wernicke encephalopathy. Wernicke encephalopathy presents with ocular palsies, confusion, and gait ataxia and is also related to a thiamine deficiency. In Wernicke-Korsakoff syndrome, lesions are always found in the mammillary bodies and the dorsomedial nuclei of the thalamus.

In addition to exhibiting an anterograde amnesia, Korsakoff patients also present with retrograde amnesia. These patients confabulate, making up stories to replace past memories they can no longer retrieve.

Klüver-Bucy Syndrome

Klüver-Bucy syndrome results from bilateral lesions of the amygdala and hippocampus. These lesions result in:

- Placidity–there is marked decrease in aggressive behavior; the subjects become passive, exhibiting little emotional reaction to external stimuli.
- Psychic blindness–objects in the visual field are treated inappropriately. For example, monkeys may approach a snake or a human with inappropriate docility.
- Hypermetamorphosis–visual stimuli (even old ones) are repeatedly approached as though they were completely new.
- Increased oral exploratory behavior–monkeys put everything in their mouths, eating only appropriate objects.
- Hypersexuality and loss of sexual preference
- Anterograde amnesia

Alzheimer Disease

- Alzheimer disease accounts for 60% of all cases of dementia. The incidence increases with age.
- **Clinical:** insidious onset, progressive memory impairment, mood alterations, disorientation, aphasia, apraxia, and progression to a bedridden state with eventual death
- Five to 10% of Alzheimer cases are hereditary, early onset, and transmitted as an autosomal dominant trait.

Lesions involve the neocortex, hippocampus, and subcortical nuclei, including forebrain cholinergic nuclei (i.e., basal nucleus of Meynert). These areas show atrophy, as well as characteristic microscopic changes. The earliest and most severely affected areas are the hippocampus and temporal lobe, which are involved in learning and memory.

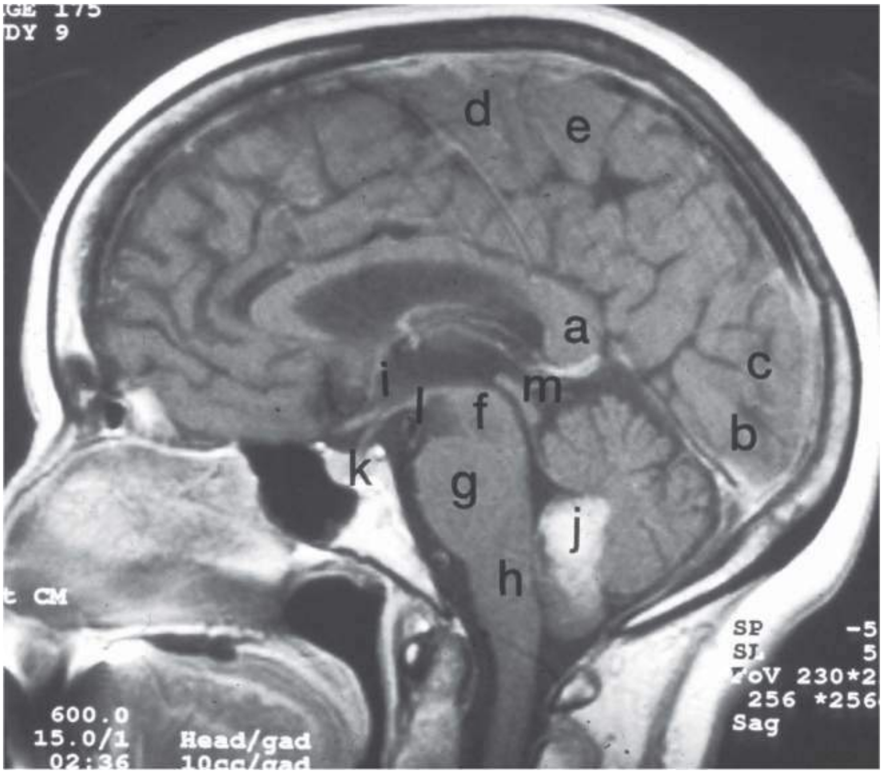


Figure III-11-2. MRI of Medial View of the Central Nervous System

(a) Corpus callosum (splenium) (b) Lingual gyrus (c) Cuneus gyrus (d) Primary motor cortex (e) Primary somatosensory cortex (f) Midbrain (g) Pons (h) Medulla (i) Hypothalamus in wall of third ventricle (j) Posterior vermis of cerebellum with intracerebral hemorrhage (k) Pituitary (l) Mammillary body (m) Pineal

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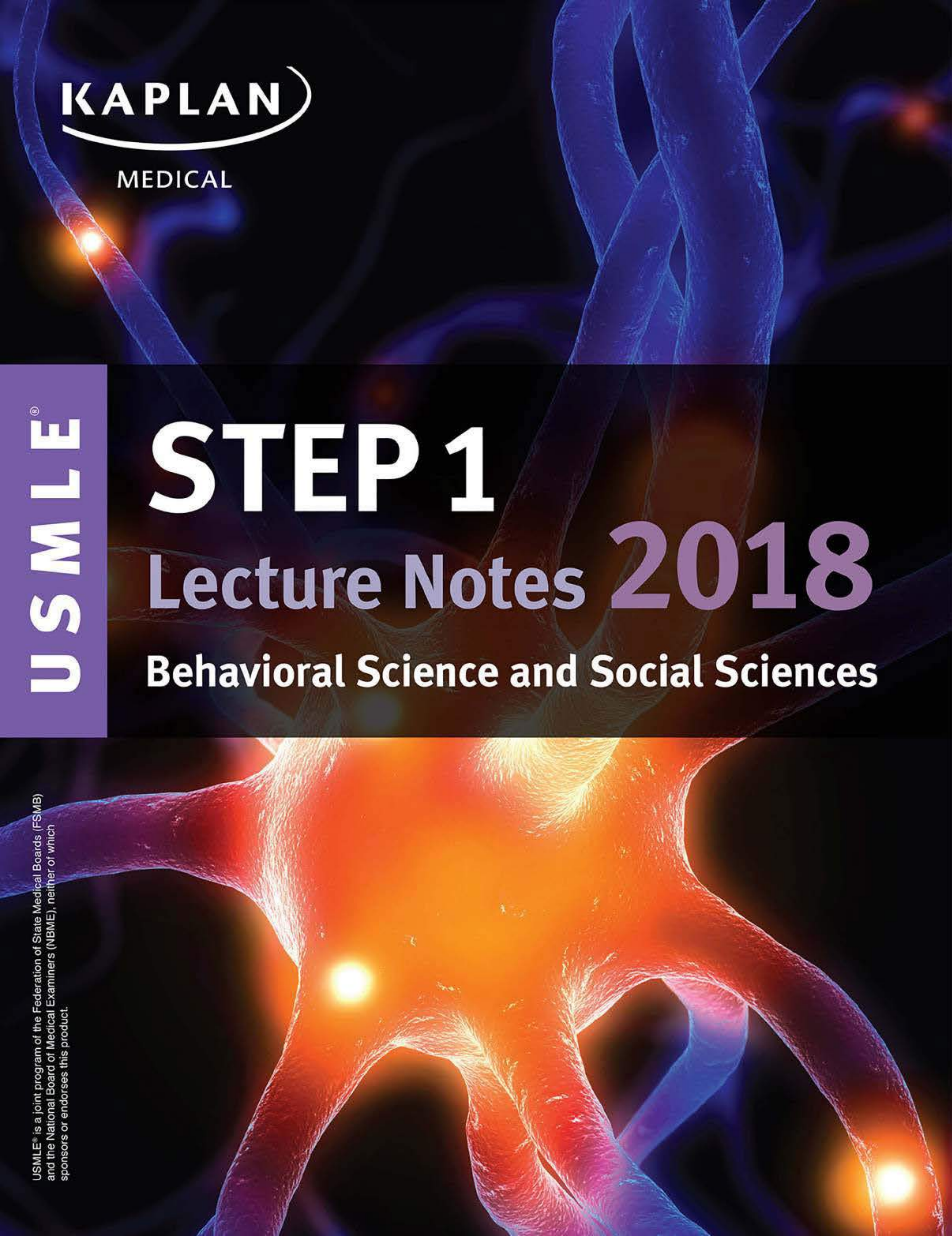

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PART I

Epidemiology and Biostatistics

Epidemiology 1

Learning Objectives

❑ Answer questions about epidemiologic measures

❑ Use knowledge of screening tests

❑ Explain information related to study designs

EPIDEMIOLOGIC MEASURES

Epidemiology is the study of the distribution and determinants of health-related states within a population. It refers to the patterns of disease and the factors that influence those patterns.

- **Endemic**: the usual, expected rate of disease over time; the disease is maintained without much variation within a region
- **Epidemic**: occurrence of disease in excess of the expected rate; usually presents in a larger geographic span than endemics (*epidemiology* is the study of epidemics)
- **Pandemic**: worldwide epidemic
- **Epidemic curve**: visual description (commonly histogram) of an epidemic curve is disease cases plotted against time; classic signature of an epidemic is a "spike" in time

The tools of epidemiology are numbers; the numbers in epidemiology are ratios converted into rates. The denominator is key: who is "at risk" for a particular event or disease state.

To determine the rate, compare the number of actual cases with the number of potential cases:

$$\frac{\text{Actual cases}}{\text{Potential cases}} = \frac{\text{Numerator}}{\text{Denominator}} = \text{RATE}$$

Rates are generally, though not always, per 100,000 persons by the Centers for Disease Control (CDC), but can be per any multiplier. (Vital statistics are usually per 1,000 persons.)

A disease may occur in a country at a regular annual rate, which makes it **endemic**. If there is a sudden rise in the number of cases in a specific month, we say that there is an **epidemic**. As the disease continues to rise and spread to other countries, it becomes a **pandemic**. Thus the terminology is related to both the number of cases and its geographical distribution.

The graph below represents the incidence of 2 diseases (cases in 100,000). Disease 1 is endemic as the rate of disease is consistent month to month with minor variation in the number of cases. Disease 2 experiences an epidemic in March and April in which the number of cases is in excess of what is expected.

January	February	March	April	May	June	July	August
3	4	3	4	4	4	3	3
5	5	8	8	5	5	5	5

Although the data is in 100,000 cases, the variation in disease 1 is still consistent when compared to disease 2.

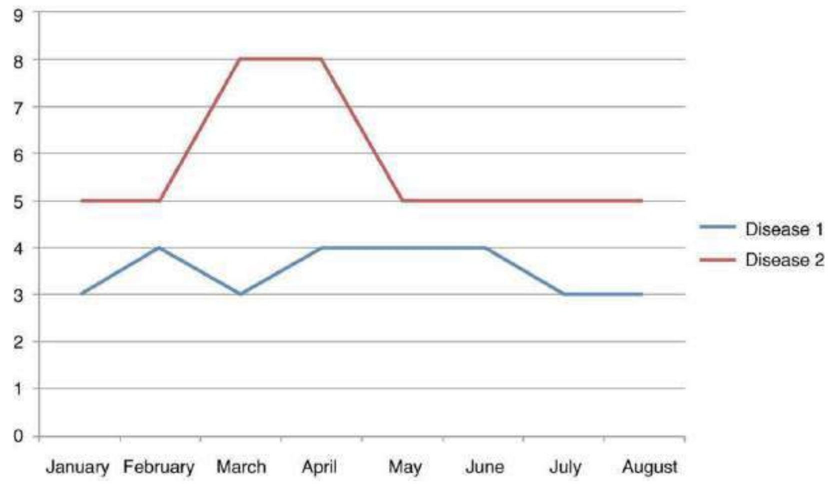


Figure 1.1 Epidemic vs. Endemic Cases

Consider the following scenario. A Japanese farmer begins to sell meat that is infected with salmonella. Within 2 days, hundreds of villagers begin to experience crampy abdominal pain. This is an example of an epidemic. The sudden rise of salmonella gastroenteritis in this village is much higher than the average incidence for the given time period.

Now what if the farmer ships 1,000 pounds of infected beef to other regions of Japan before he realizes what happened? What can one anticipate would happen? The answer is there would be no change to the endemic rate of gastroenteritis. The farmer is only shipping out 1,000 pounds of beef to a few cities nationwide. Unlike the earlier scenario which addressed the population of a village, this would be the entire nation. Assuming that every person who consumes the beef gets gastroenteritis, that number would not significantly increase the national average of cases and would therefore not significantly change the incidence of the disease nationwide.

Incidence and Prevalence

Incidence rate (IR) is the rate at which **new events** occur in a population.

- The numerator is the number of **new** events that occur in a defined period.
- The denominator is the population at risk of experiencing this new event during the same period.

$$\text{Incidence rate} = \frac{\text{Number of new events in a specified period}}{\text{Number of persons "exposed to risk" of becoming new cases during this period}} \times 10^n$$

The IR includes only **new cases** of the disease that occurred during the specified period, not cases that were diagnosed earlier. This is especially important when working with infectious diseases such as TB and malaria.

If, over the course of a year, 5 men are diagnosed with prostate cancer, out of a total male study population of 200 (with no prostate cancer at the beginning of the study period), the IR of prostate cancer in this population would be 0.025 (or 2,500 per 100,000 men-years of study).

Attack rate is the cumulative incidence of infection in a group of people observed over a period of time during an epidemic, usually in relation to food-borne illness. It is measured from the beginning of an outbreak to the end of the outbreak.

$$\text{Attack rate} = \frac{\text{Number of exposed people infected with the disease}}{\text{Total number of exposed people}}$$

Attack rate is also called *attack ratio*; consider an outbreak of Norwalk virus in which 18 people in separate households become ill. If the population of the community is 1,000, the overall attack rate is $\frac{18}{1{,}000} \times 100\% = 1.8\%$.

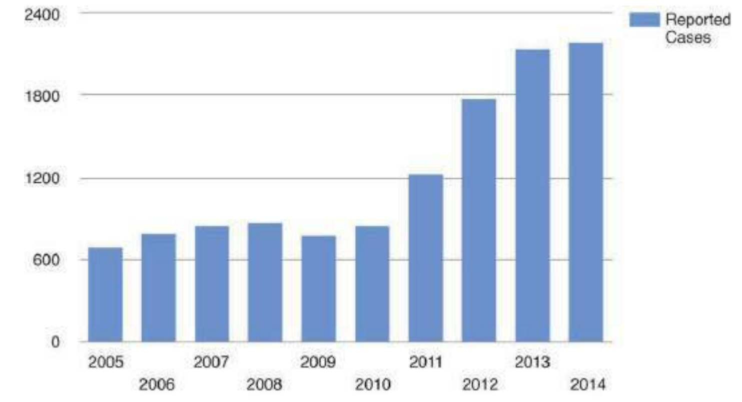


Figure 1.2 Reported Cases of Hepatitis C in the United States

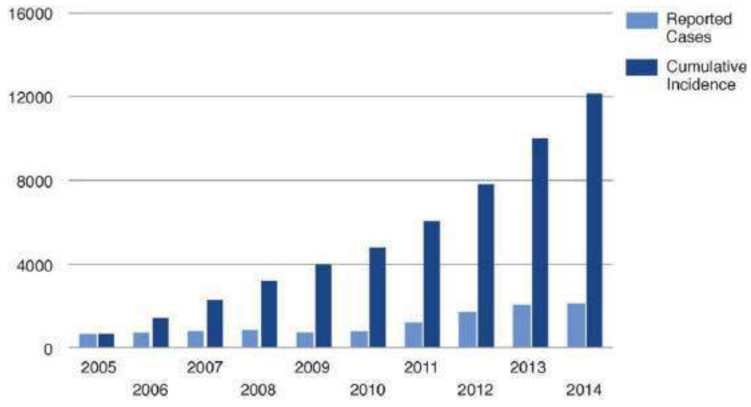


Figure 1.3 Cumulative Incidence 2005–2015

Prevalence is all persons who experience an event in a population. The numerator is all individuals who have an attribute or disease at a particular point in time (or period of time). The denominator is the population at risk of having the attribute or disease at that point in time or midway through the period.

$$\text{Prevalence} = \frac{\text{All cases of a disease at a given point / period}}{\text{Total population "at risk" for being cases at a given point / period}} \times 10^{n}$$

Prevalence, in other words, is the proportion of people in a population who have a particular disease at a specified point in time (or over a specified period of time). The numerator includes both new cases and old cases (people who remained ill during the specified point or period in time). A case is counted in prevalence until death or recovery occurs. **This makes prevalence different from incidence, which includes only new cases in the numerator.**

Prevalence is most useful for measuring the burden of chronic disease in a population, such as TB, malaria and HIV. For example, the CDC estimated the prevalence of obesity among American adults in 2001 at approximately 20%. Since the number (20%) includes all cases of obesity in the United States, we are talking about *prevalence*.

Note

Prevalence is a measurement of *all* individuals (new and old) affected by the disease at a particular time, whereas **incidence** is a measurement of the number of *new* individuals who contract a disease during a particular period of time.

Point prevalence is useful for comparing disease at different points in time in order to determine whether an outbreak is occurring. We know that the amount of disease present in a population changes over time, but we may need to know how much of a particular disease is present in a population at a single point in time ("snapshot view").

Perhaps we want to know the prevalence of TB in Community A today. To do that, we need to calculate the point prevalence on a given date. The numerator would include all known TB patients who live in Community A that day. The denominator would be the population of Community A that day.

Period prevalence, on the other hand, is prevalence during a specified period or span of time. The focus is on *chronic* conditions.

In the **"prevalence pot,"** incident (or new) cases are monitored over time. New cases join pre-existing cases to make up total prevalence.

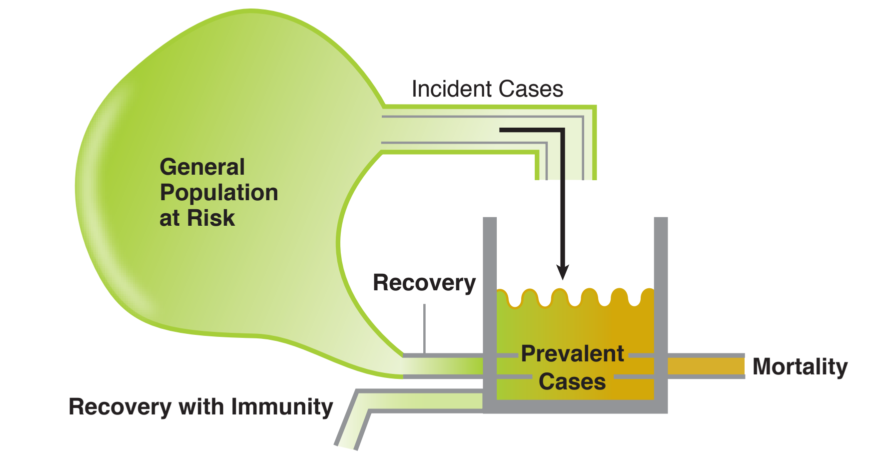


Figure 1-4. Prevalence Pot

Prevalent cases leave the prevalence pot in one of 2 ways: recovery or death.

Table 1-1. Incidence and Prevalence

What happens if:	Incidence	Prevalence
New effective treatment is initiated	no change	decrease
New effective vaccine gains widespread use	decrease	decrease
Number of persons dying from the condition increases	no change	decrease
Additional Federal research dollars are targeted to a specific condition	no change	no change
Behavioral risk factors are reduced in the population at large	decrease	decrease
Contacts between infected persons and noninfected persons are reduced		
For airborne infectious disease?	decrease	decrease
For noninfectious disease?	no change	no change
Recovery from the disease is more rapid than it was one year ago	no change	decrease
Long-term survival rates for the disease are increasing	no change	increase

Note

Morbidity rate is the rate of disease in a population at risk (for both incident and prevalent cases), while **mortality rate** is the rate of death in a population at risk (incident cases only).

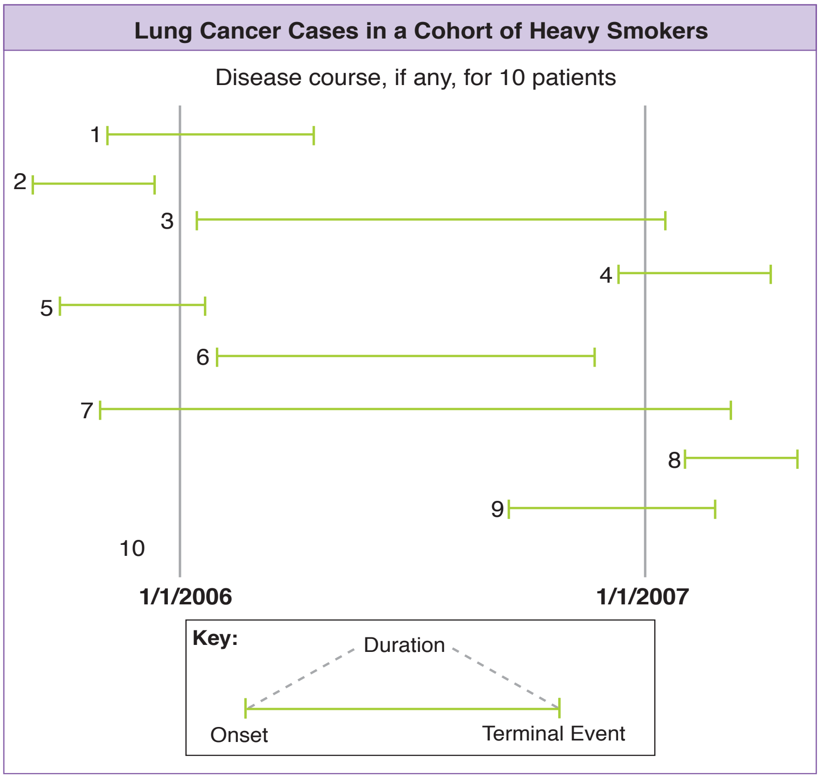


Figure 1-5. Calculating Incidence and Prevalence

Based on the graph above, calculate the following:

- **Prevalence of lung cancer from 1/1/2006–1/1/2007**
  - Number of patients who "had" lung cancer in this time period from the graph: (7)
  - Number of patients at risk in this time period: (9) [exclude patient #2 who died before the time period]
  - Prevalence: (7/9)
  - Type of prevalence: (period prevalence)
- **Incidence of lung cancer from 1/1/2006–1/1/2007**
  - Number of patients who developed lung cancer in this time period: (4)
  - Number of patients at risk in this time period: (6) [exclude patients who were already sick at the start of the time period and those who died before the time period]
  - Incidence: (4/6)

Crude, Specific, and Standardized Rates

Crude rate is the actual measured rate for a **whole population**, e.g., rate of myocardial infarction for a whole population. Use caution using the crude rate, though. Imagine that in a given city, there are a lot of older, retired people—the crude rate of myocardial infarction will appear higher even though the rate for each age group has not actually changed.

Specific rate is the actual measured rate for **subgroup of population**, e.g., "age-specific" or "sex-specific" rate. For instance, the rate of myocardial infarction among people age >65 in the population or the rate of breast cancer among the female population.

If you are provided specific rates, you can calculate the crude rate. The crude rate of an entire population is a weighted sum of each of the specific rates. The weighted specific rates that are added together is calculated in the table below.

Standardized rate (or adjusted rate) is adjusted to make groups equal on some factor, e.g., age; an "as if" statistic for comparing groups. The standardized rate adjusts or removes any difference between two populations based on the standardized variable. This allows an "uncontaminated" or unconfounded comparison.

Table 1-2. Types of Mortality Rate

Crude mortality rate	$\frac{\text{Deaths}}{\text{Population}}$	Deaths in a city in 2016 per population of the city	Crude rate of people dying in the population
Cause-specific mortality rate	$\frac{\text{Deaths from cause}}{\text{Population}}$	Deaths from lung cancer in a city in 2016 per population of the city	Specific rate of people dying from a particular disease in the population
Case-fatality rate	$\frac{\text{Deaths from cause}}{\text{Number of persons with the disease/cause}}$	Deaths from Ebola in a city per number of persons with Ebola	How likely you are to die from the disease, i.e., fatality
Proportionate mortality rate (PMR)	$\frac{\text{Deaths from cause}}{\text{All deaths}}$	Deaths from diabetes mellitus in a city per total deaths in the city	How much a disease contributes to the mortality rate, i.e., what proportion of the mortality rate is due to that disease

For example, the city of Hoboken, New Jersey has a population of 50,000. In 2016, the total number of deaths in Hoboken was 400. The number of deaths from lung cancer in Hoboken was 10, while the number of patients with lung cancer diagnosis was 30. Calculate the following:

- Mortality rate in Hoboken for 2016: (400/50,000 × 1,000)
- Cause specific mortality rate for lung cancer in Hoboken for 2016: (10/50,000 × 100,000)
- CFR for lung cancer in Hoboken in 2016: (10/30 × 100)
- PMR for lung cancer in Hoboken in 2016: (10/400 × 100)

PREVENTION

The goals of prevention in medicine are to **promote health**, **preserve health**, **restore health when it is impaired**, and **minimize suffering and distress**. These goals aim to minimize both morbidity and mortality.

- **Primary prevention** promotes health at both individual and community levels by facilitating health-enhancing behaviors, preventing the onset of risk behaviors, and diminishing exposure to environmental hazards. **Primary prevention efforts decrease disease incidence**. Examples include implementation of exercise programs and healthy food programs in schools.
- **Secondary prevention** screens for risk factors and early detection of asymptomatic or mild disease, permitting timely and effective intervention and curative treatment. **Secondary prevention efforts decrease disease prevalence**. Examples include recommended annual colonoscopy for patients age >65 and HIV testing for health care workers with needlestick injuries.
- **Tertiary prevention** reduces long-term impairments and disabilities and prevents repeated episodes of clinical illness. **Tertiary prevention efforts prevent recurrence and slow progression.** Examples include physical therapy for spinal injury patients and daily low-dose aspirin for those with previous myocardial infarction.

Consider a new healthcare bill that is being funded to help wounded war veterans gain access to prosthetic limb replacement. That would be considered tertiary prevention. The patients who would have access to the service have already been injured. The prosthetic devices would help reduce complications of amputation and help their rehabilitation. By improving quality of life and reducing morbidity, that is an implementation of tertiary prevention.

Now consider a medical student who is asked to wear a nose and mouth mask before entering the room of a patient with meningococcal meningitis. That would be considered primary prevention. Because the bacteria in this case can be spread by respiratory contact, the use of the mask will prevent the student from being exposed.

SCREENING TESTS

Screening tests help physicians to detect the presence of disease, e.g., an ELISA test for HIV, the results of which are either positive or negative for disease. The efficacy of a screening test is assessed by comparing the results to verified sick and healthy populations. For HIV, we would use a Western blot as a gold-standard.

The qualifier "true" or "false" is used to describe the correlation between the test results (positive or negative) and the disease (presence or absence).

True-positive (TP): tested positive, actually sick

- In other words, the positive result is true.

False-positive (FP): tested positive, is actually healthy

- In other words, the positive result is false.

True-negative (TN): tested negative, actually healthy

- In other words, the negative result is true.

False-negative (FN): tested negative, is actually sick

- In other words, the negative result is false.

Table 1-3. Screening Results in a 2 × 2 Table

Disease						
		Present		Absent		Totals
Screening Test Results	Positive	TP	60	FP	70	TP + FP
	Negative	FN	40	TN	30	TN + FN
	Totals	TP + FN		TN + FP		TP + TN + FP + FN

Measures of Test Performance

Sensitivity and **specificity** are measures of the test performance (and in some cases, physical findings and symptoms). They help to provide additional information in cases where it is not possible to use a gold-standard test and instead a cheaper and easier (yet imperfect) screening test is used. Think about what would happen if you called the cardiology fellow to do a cardiac catheterization (the gold standard test to diagnose acute myocardial ischemia) on a patient without first having an EKG.

Sensitivity is the probability of correctly identifying a case of disease. In other words, it is the **proportion of truly diseased persons** in the screened population who are **identified as diseased** by the screening test. This is also known as the "true positive rate."

Sensitivity = TP/(TP + FN) = true positives/(true positives + false negatives)

- Measures only the distribution of persons with disease
- Uses data from the left column of the 2 × 2 table
- Note: 1-sensitivity = false negative rate

Note

A mnemonic for the clinical use of sensitivity is **SN-N-OUT** (sensitive test-negative-rules out disease).

If a test has a **high sensitivity**, then a **negative result** indicates the **absence of the disease.** For example, temporal arteritis (TA), a large vessel vasculitis that involves branches of the external carotid artery seen in those age >50, always shows elevated ESR. So 100% of patients with TA have elevated ESR. The sensitivity of an abnormal ESR for TA is 100%. If a patient you suspect of having TA has a normal ESR, then the patient does not have TA.

If there are 200 sick people, the sensitivity of a test tells us the capacity of the test to correctly identify these sick people. If a screening test identifies 160 of them as sick (they test positive), then the sensitivity of the test is 160/200 = 80%.

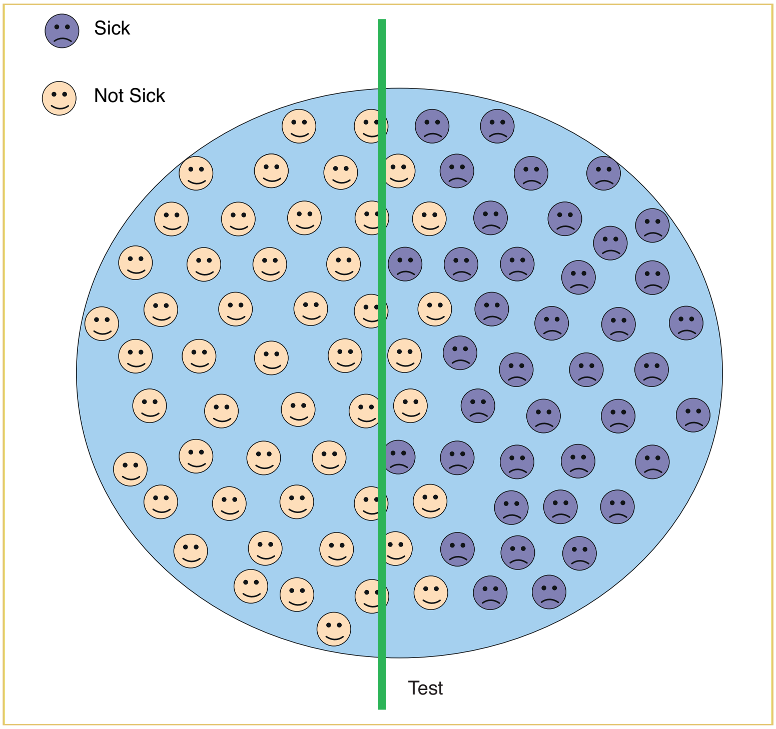


Figure 1-6. Sensitive Test

Specificity is the probability of correctly identifying disease-free persons. Specificity is the **proportion of truly nondiseased persons** who are **identified as nondiseased** by the screening test. This is also known as the "true negative rate."

Specificity = TN/(TN + FP) = true negatives/(true negatives + false positives)

- Measures only the distribution of persons who are disease-free
- Uses data from the right column of the 2 × 2 table
- Note: 1-specificity = false positive rate

If a test has a **high specificity**, then a **positive result** indicates the **existence of the disease.** For example, CT angiogram has a very high specificity for pulmonary embolism (97%). A CT scan read as positive for pulmonary embolism is likely true.

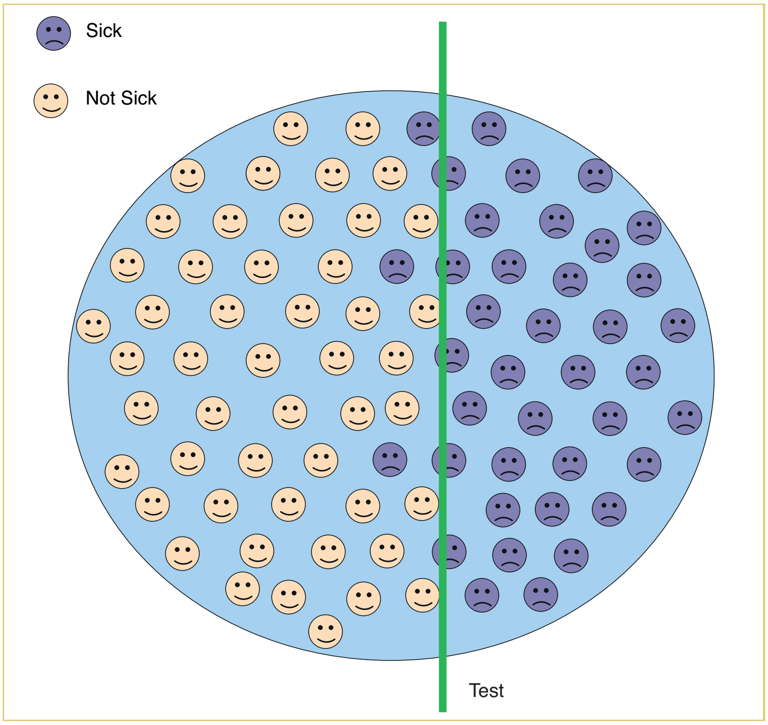


Figure 1-7. Specific Test

The separation between the sick and healthy in a given population isn't always clear; there is a measure of overlap, as in the figure above. In order to create a test that is specific and identifies only sick people as positive, it must (a) correctly identify all the healthy people and (b) not inaccurately identify healthy people as sick. In other words, the more specific the test, the fewer false-positives (i.e., healthy people incorrectly identified as sick) it will have. Specificity is therefore the capacity of a test to correctly exclude healthy people with negative test results.

Post-Test Probabilities

Positive predictive value (PPV) is the probability of disease in a person who receives a positive test result. The probability that a **person with a positive test is a true positive** (i.e., has the disease) is referred to as the "predictive value of a positive test."

PPV = TP/(TP + FP)
= true positives/(true positives + false positives)

PPV measures only the distribution of persons who receive a positive test result.

Negative predictive value (NPV) is the probability of no disease in a person who receives a negative test result. The probability that a **person with a negative test is a true negative** (i.e., does not have the disease) is referred to as the "predictive value of a negative test."

NPV = TN/(TN + FN)
= true negatives/(true negatives + false negatives)

Note

A mnemonic for the clinical use of specificity: **SP-I-N** (specific test-positive-rules in disease).

Note

For any test, there is usually a trade-off between SNNOUT and SPIN. The trade-off can be represented graphically as the screening dimension curves and ROC curves.

NPV measures only the distribution of persons who receive a negative test result.

Accuracy is the total percentage correctly selected, the degree to which a measurement, or an estimate based on measurements, represents the true value of the attribute that is being measured.

$$\text{Accuracy} = (\text{TP} + \text{TN})/(\text{TP} + \text{TN} + \text{FP} + \text{FN})$$
$$= (\text{true positives} + \text{true negatives})/\text{total screened patients}$$

Review Questions

Questions 1–3

A screening test identifies 150 out of 1,000 patients to have tuberculosis. When tested with the gold standard diagnostic test, 200 patients test positive, including 100 of those identified by the screening test.

1. What is the sensitivity of the screening test?

2. What is the specificity of the screening test?

3. What is the positive predictive value?

Answers and Explanations

1. **Answer: 50%.** Sensitivity would be true positives divided by all sick people. Only 100 of the 150 positive results were actually true, so true positives would be 100. Total sick people is 200. So we have 100/200, making sensitivity 50%.

2. **Answer: 93.75%.** Specificity would be true negatives divided by all healthy people. Only 100 of the 150 positive results were actually true, so false positives (healthy people with a positive result) would be 50. Total people is 1,000. So we have 1,000 − 200 sick, making 800 healthy. True negatives = 800 − 50 so 750. Specificity = 750/800 so 93.75%.

3. **Answer: 66%.** Positive predictive value is true positives divided by all positives. Only 100 of the 150 positive results were actually true, so true positives would be 100. The total who tested positive would be 150. Therefore, PPV is 100 divided by 150 so 66%.

Effective Prevalence

Prevalence, which is a quantified measure of disease or cases in the population, is a relevant pre-test probability of disease within the population. The **more disease in a population, i.e., high prevalence**, the greater the probability that a positive test represents actual disease (= greater PPV). The **less disease in a population, i.e., lower prevalence**, the higher the probability that a negative result is true (= greater negative predictive value).

Consider this example: Among 80-year-old diabetic patients, the prevalence of kidney failure is higher than in the general population. This increased prevalence makes a physician more likely to believe the results of a screening test that shows kidney failure for an 80-year-old diabetic patient. We intuitively understand that the PPV is higher because this cohort of patients has a higher prevalence of disease.

Conversely, if a 15-year-old girl tests positive for a myocardial infarction, a physician will find the results strange and will thus repeat the test to confirm the positive result is not a false-positive. That is because the prevalence of myocardial infarction among teenage girls is so low that a positive result is more likely to be a mistake than a case of an actual myocardial infarction. In a teenage girl, a negative result for myocardial infarction is more likely to be true (high negative predictive value) because there is a very low prevalence of disease in this age group population.

Incidence is a measure of new cases in a population. Increasing the incidence would have no effect on sensitivity or PPV because a screening test can only detect the current presence or absence of disease, not its onset.

Prevalence has no effect on the sensitivity or specificity of a test. Those are metrics of the test and can be changed only by changing the test itself.

Double Hump Graph

In the graph below, which cutoff point provides optimal sensitivity?

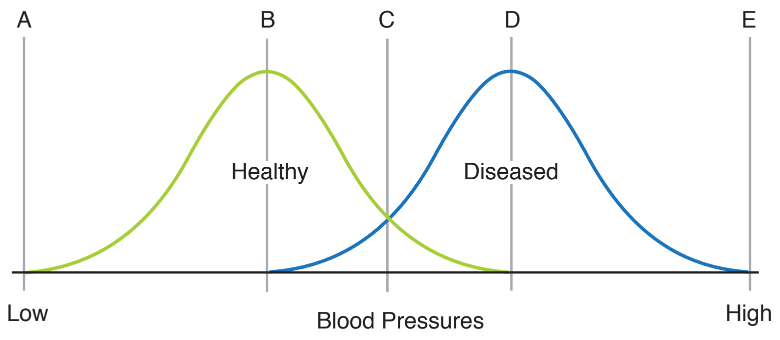


Figure 1-8. Healthy and Diseased Populations Along a Screening Dimension

Cutoff point B correctly identifies all the sick patients. It has the highest sensitivity (identifies all the sick patients). Cutoff D would be the most specific test (it identifies only sick people). Cutoff C where the 2 curves intersect is the most accurate. Note, the point of optimum sensitivity equals the point of optimum negative predictive value, while the point of optimum specificity equals the point of optimum positive predictive value.

Consider another example. Which of the following curves indicates the best screening test?

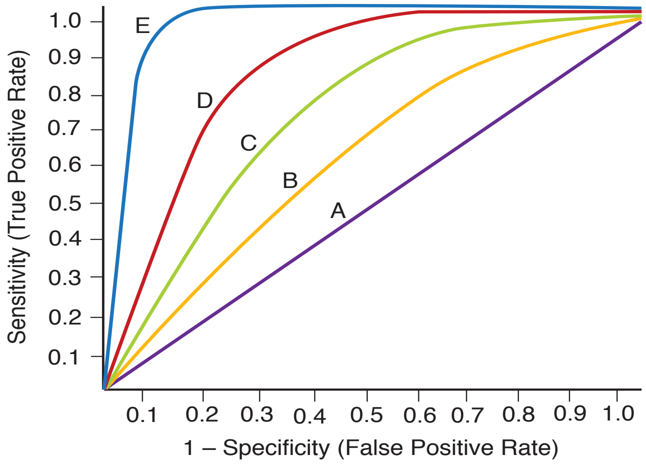


Figure 1-9. Receiver Operating Characteristic (ROC) Curves

Curve E achieves the highest sensitivity (*y*-axis) without including too many false-positives (*x*-axis).

STUDY DESIGNS

Bias in Research

Bias in research is a deviation from the truth of inferred results. It can be done intentionally or unintentionally.

Reliability is the ability of a test to **measure something consistently**, either across testing situations (test-retest reliability), within a test (split-half reliability), or across judges (inter-rater reliability). Think of the clustering of rifle shots at a target (precision).

Validity is the degree to which a test **measures that which was intended**. Think of a marksman hitting the bull's-eye. Reliability is a necessary, but insufficient, condition for validity (accuracy).

Types of bias

When there is **selection bias** (sampling bias), the sample selected is **not representative** of the population. Examples:

- Predicting rates of heart disease by gathering subjects from a local health club
- Using only hospital records to estimate population prevalence (Berkson bias)
- Including people in a study who are different from those who are not included (nonrespondent bias)
- **Solution**: random, independent sample; weight data

When there is **measurement bias**, information is gathered in a manner that **distorts the information**. Examples:

- Measuring patient satisfaction with their physicians by using leading questions, e.g., "You don't like your doctor, do you?"
- Subjects' behavior is altered because they are being studied; this is only a factor when there is no control group in a prospective study (Hawthorne effect).
- **Solution**: have a control group

When there is **experimenter expectancy** (Pygmalion effect), **experimenters' expectations are inadvertently communicated** to subjects, who then produce the desired effects. **Solution**: use **double-blind** design, where neither the subject nor the investigators know which group receives the intervention.

Lead-time bias gives a **false estimate of survival rates**, e.g., patients seem to live longer with the disease after it is uncovered by a screening test. Actually, there is no increased survival, but because the disease is discovered sooner, patients who are diagnosed *seem* to live longer. **Solution**: use life-expectancy to assess benefit.

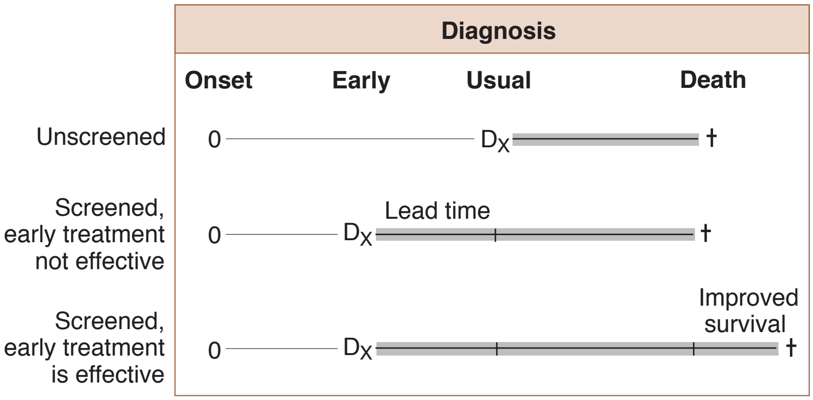


Figure 1-8. Diagnosis, Time, and Survival

When there is **recall bias**, subjects **fail to accurately recall events** in the past. For example: "How many times last year did you kiss your mother?" This is a likely problem in retrospective studies. **Solution**: confirmation.

When there is **late-look bias**, individuals with **severe disease are less likely to be uncovered** in a survey **because they die first**. For example, a recent survey found that persons with AIDS reported only mild symptoms. **Solution**: stratify by disease severity.

When there is **confounding bias**, the factor being examined **is related to other factors of less interest**. Unanticipated factors obscure a relationship or make it seem like there is one when there is not. More than one explanation can be found for the presented results. For example, compare the relationship between exercise and heart disease in 2 populations when one population is younger and the other is older. Are differences in heart disease due to exercise or to age? **Solution**: combine the results from multiple studies, meta-analysis.

When there is **design bias**, parts of the study **do not fit together** to answer the question of interest. The most common issue is a non-comparable control group. For example, compare the effects of an anti-hypertensive drug in hypertensives versus normotensives. **Solution**: random assignment, i.e., subjects assigned to treatment or control group by a random process.

Table 1-4. Type of Bias in Research

Type of Bias	Definition	Important Associations	Solutions
Selection	Sample not representative	Berkson's bias, nonrespondent bias	Random, independent sample
Measurement	Gathering the information distorts it	Hawthorne effect	Control group/placebo group
Experimenter expectancy	Researcher's beliefs affect outcome	Pygmalion effect	Double-blind design
Lead-time	Early detection confused with increased survival	Benefits of screening	Measure "back-end" survival
Recall	Subjects cannot remember accurately	Retrospective studies	Multiple sources to confirm information

Type of Bias	Definition	Important Associations	Solutions
Late-look	Severely diseased individuals are not uncovered	Early mortality	Stratify by severity
Confounding	Unanticipated factors obscure results	Hidden factors affect results	Multiple studies, good research design
Design	Parts of study do not fit together	Non-comparable control group	Random assignment

TYPES OF RESEARCH STUDIES

Observational Study

In an observational study, nature is allowed to take its course, i.e., there is no intervention.

- **Case report:** brief, objective report of a clinical characteristic/outcome from a single clinical subject or event, $n = 1$, e.g., 23-year-old man with treatment-resistant TB; there is no control group
- **Case series report:** objective report of a clinical characteristic/outcome from a group of clinical subjects, $n > 1$, e.g., patients at local hospital with treatment-resistant TB; there is no control group
- **Cross-sectional study:** the presence or absence of disease (and other variables) are determined in each member of the study population or representative sample at a particular time; co-occurrence of a variable and the disease can be examined
  - Disease prevalence, not incidence, is recorded
  - Cannot usually determine temporal sequence of cause and effect, e.g., who in the community now has treatment-resistant TB
- **Case-control study:** a group of people with the disease is identified and compared with a suitable comparison group without the disease; almost always retrospective, e.g., compares cases of treatment-resistant TB with those of nonresistant TB
  - Cannot usually assess incidence or prevalence of disease, but it can help determine causal relationships
  - Very useful for studying conditions with very low incidence or prevalence
- **Cohort study:** population group of those who have been **exposed to risk factor** is identified and followed over time and **compared with a group not exposed to the risk factor**. Outcome is disease incidence in each group, e.g., following a prison inmate population and marking the development of treatment-resistant TB
  - Prospective, meaning that subjects are tracked forward in time
  - Can determine incidence and causal relationships, and must follow population long enough for incidence to appear

Note

- Random error is unfortunate but okay and expected (a threat to reliability).
- Systematic error is bad and biases result (a threat to validity).

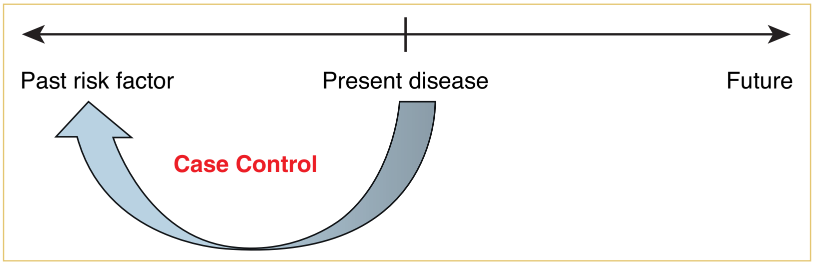


Figure 1-9. Retrospective Study

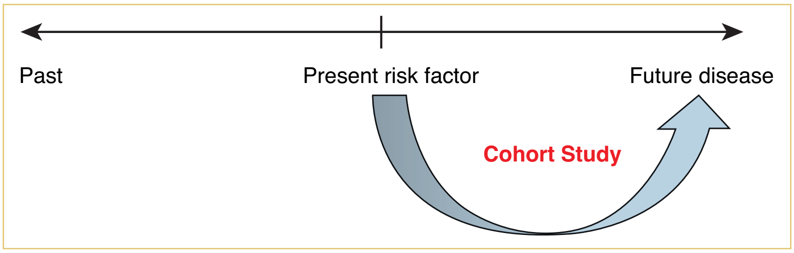


Figure 1-10. Prospective Study

Cohort Study		
Risk Factor	**Disease**	**No Disease**
	60 A	240 B
No Risk Factor	60 C	540 D

Cohort Study

Relative risk (RR) is a comparative probability asking, “How much more likely?" To find it, calculate the IR of the **exposed group divided by** the IR of the **unexposed group**. How much greater chance does one group have of contracting the disease compared with the other group?

Attributable risk (AR) is a comparative probability asking “How many more cases in one group?” To find it, calculate the IR of **exposed group minus** the IR of the **unexposed group**.

Note: Both relative and attributable risk tell us if there are differences, but they do not tell us why those differences exist.

Interpretation: for every 100 people treated, one case will be prevented.

Let's first consider **RR**. If we compare a group of 100 children who live near a chemical plant (risk factor) to a group of 100 children who do not (no risk factor), and follow them over time to see who develops asthma, we can calculate how much more likely it is for those exposed to the risk factor to develop disease, i.e., RR. In this example, say 20 children near the chemical plant and 5 children not living near the plant all develop asthma.

$$\text{RR} = \frac{\text{Incidence in exposed group (risk factor)}}{\text{Incidence in unexposed group (no risk factor)}}$$

$$= \frac{20}{100} \div \frac{5}{100} = 4$$

Interpretation: a child living near the chemical plant is 4x more likely to develop asthma than a child not living near the plant

Now let's consider **AR**. Are all 20 cases among those living near the plant due to the proximity of the plant? We know that 5 children developed asthma even though they did not live next to plant, meaning that some of the 20 cases are not necessarily due to the risk factor itself (in this case, the chemical plant).

How many of the 20 cases are due to the risk factor or, in other words, are attributable to the risk factor?

AR = Incidence in exposed group – Incidence in unexposed group

$$\frac{20}{100} - \frac{5}{100} = \frac{15}{100}$$

Interpretation: for every 100 children exposed to the risk factor, 15 cases are attributable to the risk factor itself; in other words, when we expose 100 children, 15 cases of asthma will be caused by the exposure.

So what is the **NNH**? NNH is the inverse of the attributable risk.

$$= \frac{100}{15} = 6.66 = 7 \text{ (always round up)}$$

Interpretation: for every 7 people exposed to the risk factor, there will be 1 case.

Case Control Study

For a case-control study, use **odds ratio (OR)**, which looks at the increased odds of getting a disease with exposure to a risk factor versus nonexposure to that factor. Find the odds of **exposure for cases divided by** odds of **exposure for controls**, e.g., the odds that a person with lung cancer was a smoker versus the odds that a person without lung cancer was a smoker.

Table 1-5. Case-Control Study: Lung Cancer and Smoking

	Lung Cancer	No Lung Cancer
Smokers	659 (A)	984 (B)
Nonsmokers	25 (C)	348 (D)

$$\text{Odds ratio} = \frac{A/C}{B/D} = \frac{AD}{BC}$$

Use OR = AD/BC as the working formula. For the above example:

$$OR = \frac{AD}{BC} = \frac{659 \times 348}{984 \times 25} = 9.32$$

Interpretation: the odds of having been a smoker are over 9x greater for someone with lung cancer compared with someone without lung cancer.

Odds ratio does not so much predict disease as it does estimate the strength of a risk factor.

How would you analyze the data from the following case-control study?

Case-Control Study: Colorectal Cancer and Family History

	No Colorectal Cancer	Colorectal Cancer	TOTALS
Family History of Colorectal Cancer	120	60	180
No Family History of Colorectal Cancer	200	20	220
TOTALS	320	80	400
ANSWER:	$\frac{AD}{BC}$	$\frac{(60)(200)}{(120)(20)}$	OR = 5.0

Interpretation: the odds of having a family history of colorectal cancer are 5x greater for those who have the disease than for those who do not.

Table 1-6. Differentiating Observational Studies

Characteristic	Cross-Sectional Studies	Case-Control Studies	Cohort Studies
Time	One time point	Retrospective	Prospective
Incidence	NO	NO	YES
Prevalence	YES	NO	NO
Causality	NO	YES	YES
Role of disease	Prevalence of disease	Begin with disease	End with disease
Assesses	Association of risk factor and disease	Many risk factors for single disease	Single risk factor affecting many diseases
Data analysis	*Chi*-square to assess association	Odds ratio to estimate risk	Relative risk to estimate risk

Clinical Trials

Researchers design clinical trials to answer specific research questions related to a medical product. A **control group** (often the *placebo* group) will includes subjects who **do not receive the intervention under study**, used as a **source of comparison** to be certain the experiment group is being affected by the intervention and not by other factors. Control group subjects must be **as similar as possible to intervention group** subjects.

For a medical product to receive approval by the Food and Drug Administration (FDA), 3 phases must be passed.

- **Phase 1**: testing safety in healthy volunteers
- **Phase 2**: testing protocol and dose levels in a small group of patient volunteers
- **Phase 3** (**definitive test**): testing efficacy and occurrence of side effects in a larger group of patient volunteers

Post-FDA approval, marketing surveys will collect reports of drug side effects among populations commonly using the product.

In a **randomized controlled clinical trial (RCT)**, subjects are **randomly allocated** into “intervention” and “control” groups to receive or not receive an experimental/preventive/therapeutic procedure or intervention. This is generally regarded as the **most scientifically rigorous** type of study available in epidemiology.

A **double-blind RCT** is the type of study **least subject to bias**, but also the **most expensive** to conduct. Double-blind means that neither subjects nor researchers know whether the subjects are in the treatment or comparison group. A double-blind study has 2 types of control groups:

- Placebos (25–40% often show improvement in placebo group)
- Standard of care (current treatment versus new treatment)

A **community trial** is an experiment in which the unit of allocation to receive a preventive or therapeutic regimen is an **entire community or political subdivision**. Does the treatment work in real-world circumstances?

A **cross-over study** is one in which, for ethical reasons, no group involved can remain untreated. **All subjects receive the intervention** but at different times (making recruitment of subjects easier). Assume double-blind design. For example, an AZT trial, where group A receives AZT for 3 months while group B is the control. For the second 3 months, group B receives AZT and group A is the control.

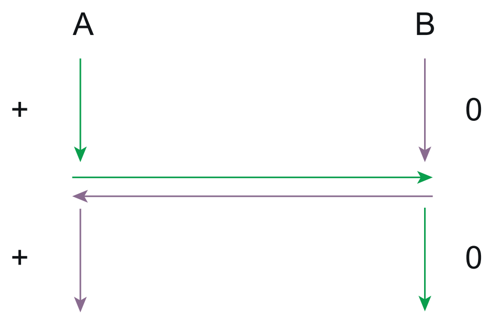


Figure 1-11. Cross-Over Study

Biostatistics 2

Learning Objectives

- ❏ Demonstrate understanding of key probability rules
- ❏ Summarize data
- ❏ Solve problems using inferential statistics
- ❏ Use knowledge of nominal, ordinal, interval, and ratio scales
- ❏ Answer questions about statistical tests

KEY PROBABILITY RULES

Independent Events

Events are **independent** if the **occurrence of one tells you nothing about the occurrence of the other**. Combine probabilities for independent events by **multiplication**.

The issue here is the intersection of 2 sets; e.g., if the chance of having blond hair is 0.3 and the chance of having a cold is 0.2, the chance of meeting a blond-haired person with a cold is: $0.3 \times 0.2 = 0.06$ (or 6%).

If events are **nonindependent**, multiply the probability of one event by the probability of the second, assuming that the first has occurred; e.g., if a box has 5 white balls and 5 black balls, the chance of picking 2 black balls is:

$$\frac{5}{10} \times \frac{4}{9} = 0.5 \times 0.44 = 0.22 \text{ (or 22\%)}$$

Mutually Exclusive Events

Events are mutually exclusive if the **occurrence of one event precludes the occurrence of the other**. Combine probabilities for mutually exclusive outcomes by **addition**.

The issue here is the union of two sets; e.g., if a coin lands on heads, it cannot be tails; the events are **mutually exclusive**. If a coin is flipped, the chance that it will be either heads or tails is $0.5 + 0.5 = 1.0$ (or 100%).

If 2 events are **not mutually exclusive**, add them together and subtract out the multiplied probabilities to get the combination of probabilities. For example, if the chance of having diabetes is 10% and the chance of being obese is 30%, the chance of meeting someone who is obese or has diabetes or both is $0.1 + 0.3 - (0.1 \times 0.3) = 0.37$ (or 37%).

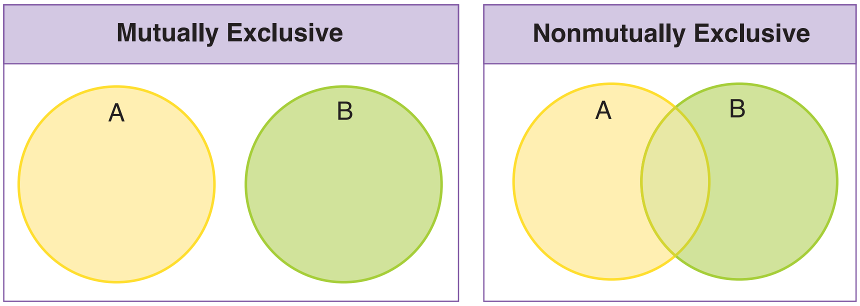


Figure 2-1. Venn Diagram of Mutually Exclusive and Nonmutually Exclusive Events

Review Questions

1. If the prevalence of diabetes is 10%, what is the chance that 3 people selected at random from the population will all have diabetes?

2. Chicago has a population of 10,000,000. If 25% of the population is Latino, 30% is African American, 5% is Arab American, and 40% is of European extraction, how many people in Chicago are classified as other than of European extraction?

3. At age 65, the probability of surviving for the next 5 years is 0.8 for a white man and 0.9 for a white woman. For a married couple who are both white and age 65, the probability that the wife will be a living widow 5 years later is:

   A. 90%
   B. 72%
   C. 18%
   D. 10%
   E. 8%

4. If the chance of surviving for 1 year after being diagnosed with prostate cancer is 80% and the chance of surviving for 2 years after diagnosis is 60%, what is the chance of surviving for 2 years after diagnosis, given that the patient is alive at the end of the first year?

   A. 20%
   B. 48%
   C. 60%
   D. 75%
   E. 80%

Answers and Explanations

1. **Answer: 0.001.** $0.1 \times 0.1 \times 0.1 = 0.001$

2. **Answer: 6,000,000.** $25\% + 30\% + 5\% = 60\%$. $60\% \times 10{,}000{,}000 = 6{,}000{,}000$

3. **Answer: C.** You're being asked for the joint probability of independent events; therefore, the probabilities are multiplied. Chance of the wife being alive: 90%, and chance of the husband being dead: $100\% - 80\% = 20\%$. Therefore, $0.9 \times 0.2 = 18\%$.

4. **Answer: D.** The question tests knowledge of "conditional probability." Out of 100 patients, 80 are alive at the end of 1 year and 60 at the end of 2 years. The 60 patients alive after 2 years are a subset of those that make it to the first year. Therefore, $60/80 = 75\%$.

DESCRIPTIVE STATISTICS

Distributions

Statistics deals with the world as *distributions*. These distributions are summarized by a **central tendency** and **variation around that center**. The most important distribution is the **normal** or **Gaussian curve**. This "bell-shaped" curve is **symmetric**, with one side the mirror image of the other.

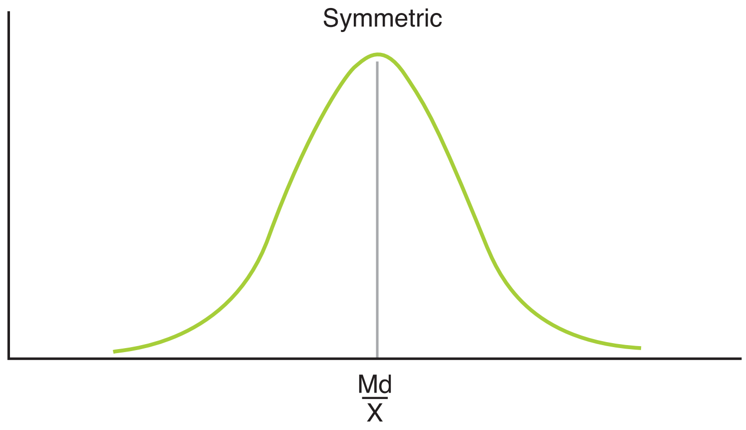


Figure 2-2. Measures of Central Tendency

Measures of central tendency

Central tendency describes a single value which attempts to describe a set of data by identifying the central (or middle) value within that set. (Colloquially, measures of central tendency are often called *averages*.) There are several valid measures:

- **Mean ($\bar{X}$)** (or *average*): sum of the values of the observations divided by the numbers of observations
- **Median (Md)**: point on the scale which divides a group into 2 parts (upper and lower half); the measurement below which half the observations fall is *50th percentile*
- **Mode**: **most frequently occurring value** in a set of observations

Given the distribution of numbers: 3, 6, 7, 7, 9, 10, 12, 15, 16, the **mode is 7**, the **median is 9**, and the **mean is 9.4**.

Not all curves are normal; sometimes the curve is **skewed** positively or negatively.

- A **positive skew** has the tail to the right, and the mean greater than the median.
- A **negative skew** has the tail to the left, and the median greater than the mean.

For skewed distributions, the **median** is a better representation of central tendency than is the mean.

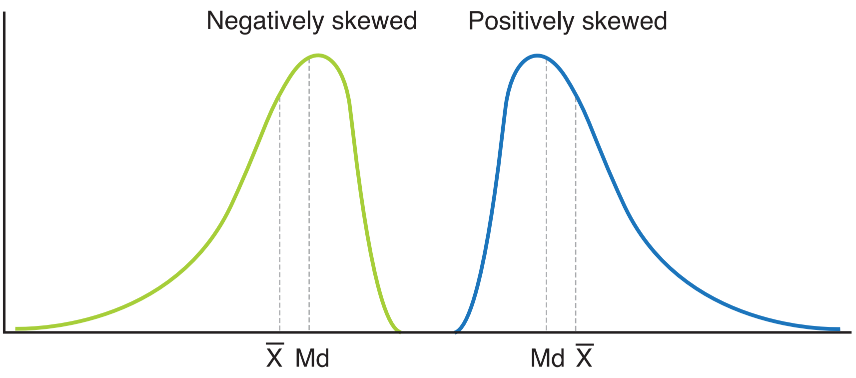


Figure 2-3. Skewed Distribution Curves

Measures of variability

The simplest measure of variability in statistics is the **range**, the difference between the highest and the lowest score. However, the range is unstable and can change easily. A more stable and more useful measure of dispersion is the **standard deviation (S** or **SD)**. To calculate the SD:

- First subtract the mean from each score to obtain **deviations from the mean**. This will give us both positive and negative values.
- Then square the deviations to make them all positive.
- Add the squared deviations together and divide by the number of cases.
- Take the square root of this average, and the result is the SD:

$$s = \sqrt{\frac{\sum\left(X - \bar{X}\right)^2}{n - 1}}$$

The square of the SD (s^2) equals the **variance**.

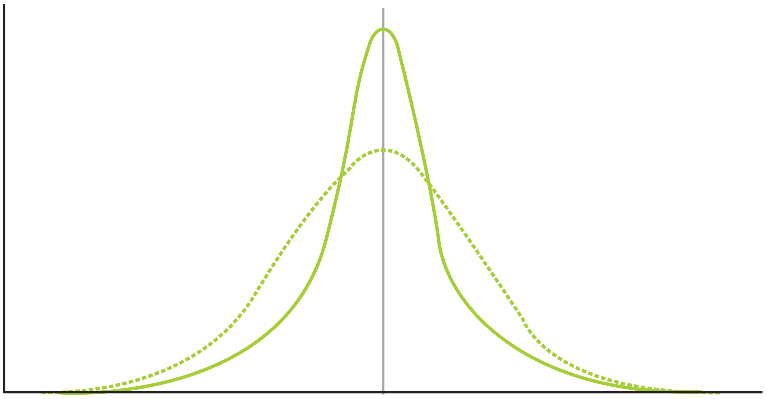


Figure 2-4. Two Normal Curves with the Same Mean but Different SDs

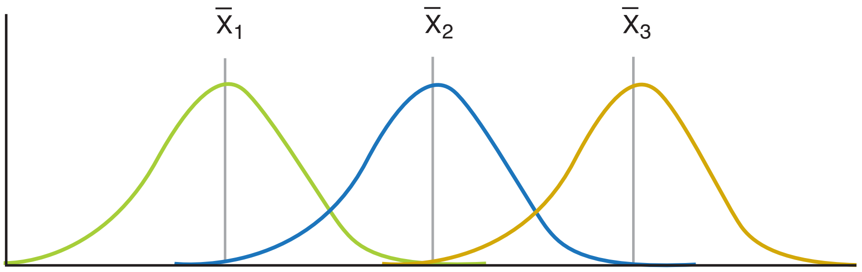


Figure 2-5. Three Normal Curves with Same SD but Different Means

Note

On the exam you will not be asked to calculate SD and variance, but you will need to understand how they relate to the normal curve. Also, be able to combine the given SD constants to answer basic questions.

In any normal curve, a constant proportion of the cases fall within 1, 2, and 3 SDs of the mean: within 1 SD 68%; within 2 SDs 95.5%; and within 3 SDs 99.7%.

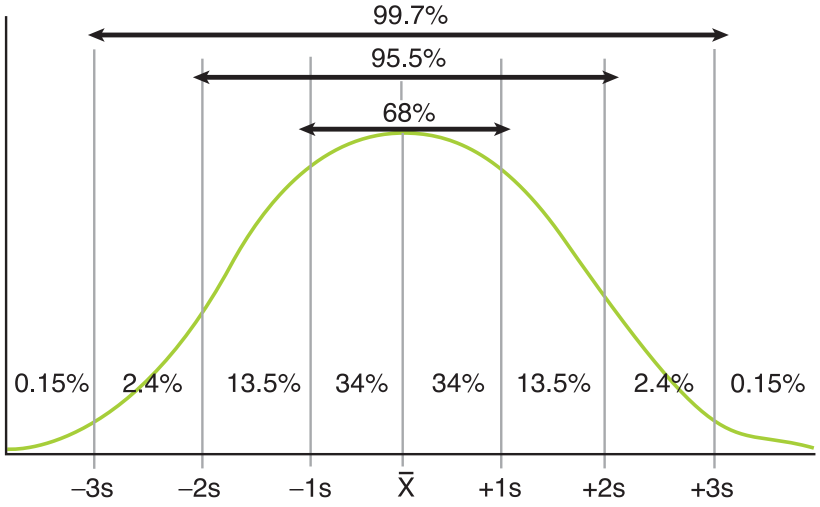


Figure 2-6. Percentage of Cases within 1, 2, and 3 SDs of the Mean in a Normal Distribution

Review Questions

5. In a normal distribution curve, what percent of the cases are below 2 SDs below the mean?
6. In a normal distribution curve, what percent of the cases are above 1 SD below the mean?
7. A student who scores at the 97.5 percentile falls where on the curve?
8. A student took 2 tests: On test A his results were score 45%, mean 30%, and SD 5%. On test B the results were score 60%, mean 40%, and SD 10%. On which test did the student do better, relative to his classmates?

Answers and Explanations

5. **Answer:** 2.5%

6. **Answer:** 84%

7. **Answer:** 2 SDs above the mean

8. **Answer:** On test A, he scored 3 SDs above the mean versus only 2 SDs above the mean for test B.

INFERENTIAL STATISTICS

The purpose of inferential statistics is to designate **how likely it is that a given finding is simply the result of chance**. Inferential statistics would not be necessary if investigators could study all members of a population. However, because that can rarely be done, using select samples that are representative of an entire population allows us to **generalize the results from the sample to the population**.

Confidence Interval

Confidence interval is a way of admitting that any measurement from a sample is only an **estimate** of the population, i.e., although the estimate given from the sample is likely to be close, the true values for the population may be above or below the sample values. A confidence interval **specifies how far above or below a sample-based value the population value lies** within a given range, from a possible high to a possible low. Reality, therefore, is most likely to be somewhere within the specified range.

To calculate the confidence interval: **study result +/– Z score × standard error**

Study result might be a mean, a relative risk or any other relevant measure that is the result of the data from the study itself. **Z score** depends on the level of confidence required. In medicine, the requirement is **at least a 95% confidence interval**. So the options are as follows:

- Z score for 95% confidence interval = 1.96 = 2
- Z score for 99% confidence interval = 2.58 = 2.5

While the SD measures the variability within a single sample, the **standard error** estimates the variability *between* samples. The standard error is usually provided. The smaller the standard error, the better and more precise the study. The standard error is affected by 2 factors: the SD and the sample size (n). The greater the SD, (high variation in the data), the greater the standard error, and the larger the sample size, the smaller the standard error.

$$\textbf{Standard Error} = \frac{SD}{\sqrt{n}}$$

Suppose 100 students in the 9th grade have just taken their final exam, and the mean score was 64% with SD 15. The 95% confidence interval of the mean for 9th grade students in the population would be as follows:

- Mean = 65
- Z score for 95% confidence = 2 (rounded up Z score)
- SD = 15
- Sample size = 100

$$\text{Plug in the numbers: Mean } +/- \text{Z score} \left(\frac{SD}{\sqrt{n}}\right)$$

$$\text{or } 65 +/- 2(15/10) = 65 +/- 3$$

What this means is that we are 95% sure that the mean score of 9th graders in the population will fall somewhere between 62 and 68.

Assuming the graph below presents 95% confidence intervals, which groups, if any, are statistically different from each other?

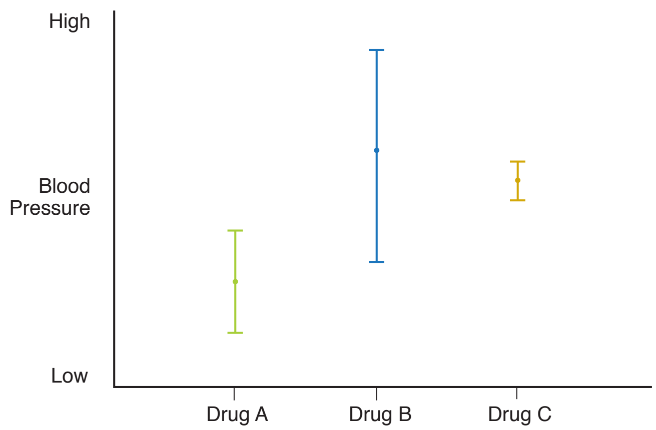


Figure 2-7. Blood Pressure at End of Clinical Trial for 3 Drugs

When comparing 2 groups, any overlap of confidence intervals means the groups are not significantly different. If the graph represents 95% confidence intervals, drugs B and C are no different in their effects; drug B is no different from drug A and drug A has a better effect than drug C.

For the confidence interval for **relative risk and odds ratios**, consider the following: **If the given confidence interval contains 1.0, then there is no statistically significant effect of exposure**. For example:

Relative Risk	Confidence Interval	Interpretation
1.77	(1.22 – 2.45)	Statistically significant (increased risk)
1.63	(0.85 – 2.46)	NOT statistically significant (risk is the same)
0.78	(0.56 – 0.94)	Statistically significant (decreased risk)

If **RR** >**1.0**, then subtract 1.0 and read as percent increase. So 1.77 means one group has 77% more cases than the other. If **RR** <**1.0**, then subtract from 1.0 and read as reduction in risk. So 0.78 means one group has a 22% reduction in risk.

Statistical Inference

The goal of science is to define reality. Think of statistics as the referee in the game of science. We have all agreed to play the game according to the judgment calls of the referee, even though we know the referee can, and will, be wrong at times. The basic steps of statistical inference are as follows:

1. Define the **research question**: What are you trying to show?
2. Define the **null hypothesis** (generally the opposite of what you hope to show). Null hypothesis says that the **findings are the result of chance or random factors**. If you want to show that a drug works, the null hypothesis will be that the drug does NOT work. The **alternative hypothesis** says what is left after defining the null hypothesis. In this example, that the drug does actually work.

There are 2 types of null hypothesis:

- **One-tailed**, i.e., directional or "one-sided," such that one group is greater than or less than the other (Group A is not less than Group B, or Group A is not greater than Group B)
- **Two-tailed**, i.e., nondirectional or "two-sided," such that 2 groups are not the same (Group A ≠ Group B)

Once the data are collected and analyzed by the appropriate statistical test, the **hypothesis testing** is begun. How to run these tests is not tested on the exam, but you may need to be able to interpret results of statistical tests with which you are presented.

To interpret output from a statistical test, focus on the ***p*-value**. The term *p*-value refers to 2 things. In its first sense, the *p*-value is a standard against which we compare our results. In the second sense, the *p*-value is a result of computation. The **computed *p*-value is compared with the *p*-value criterion to test statistical significance**. If the computed value is less than the criterion, we have achieved statistical significance. In general, the smaller the *p* the better.

The *p*-value criterion is traditionally set at $p \leq 0.05$. (Assume these are the criteria if no other value is explicitly specified.) Using this standard:

- If $p \leq 0.05$, reject the null hypothesis (reached statistical significance).
- If $p > 0.05$, do not reject the null hypothesis (has not reached statistical significance).

Note

We never accept the null hypothesis. We either reject it or fail to reject it. Saying we do not have sufficient evidence to reject it is not the same as being able to affirm that it is true.

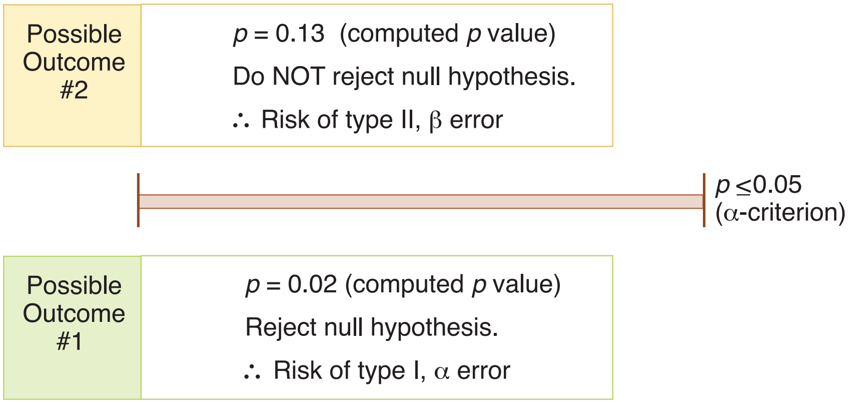


Figure 2-8. Making Decisions Using *p*-Values

- If $p = 0.02$, reject the null hypothesis, i.e., decide that the drug works.
- If $p = 0.13$, fail to reject the null hypothesis, i.e., decide that the drug does not work.

Note

A **type I error (error of commission)** is generally considered **worse** than a type II error (error of omission).

- If the null hypothesis is rejected, there is no chance of a type II error.
- If the null hypothesis is not rejected, there is no chance of a type I error.

Types of error

If we do reject the null hypothesis, we are still not certain we are correct, i.e., the results given by the sample may be inconsistent with the full population. If that is true, any decision made on the basis of the sample could be in error.

Two types of errors can be made:

- **Type I error (α error): rejecting the null hypothesis when it is really true**
  - This type of error assumes a statistically significant effect on the basis of the sample when there is none in the population, e.g., asserting that the drug works when it doesn't.
  - The chance of type I error is given by the *p*-value; if $p = 0.05$, then the chance of a type I error is 5 in 100, or 1 in 20 if we reject the null hypothesis based on the evidence of the data.
- **Type II error (β error): failing to reject the null hypothesis when it is really false**
  - This type of error declares no significant effect on the basis of the sample when there really is one in the population, e.g., asserting the drug does not work when it really does.
  - The chance of a type II error cannot be directly estimated from the *p*-value.

The ***p*-value** here does a few things:

- Provides criterion for making decisions about the null hypothesis
- Quantifies the chance that a decision to reject the null hypothesis will be wrong
- Tells statistical significance, not clinical significance or likelihood of benefit

The *p*-value **does not** tell us the following:

- Chance that an individual patient will benefit
- Percentage of patients who will benefit
- Degree of benefit expected for a given patient

Statistical power

In statistics, **power** is the capacity to detect a difference if there is one. Just as increasing the power of a microscope makes it easier to see what is going on in histology, increasing statistical power allows us to detect what is happening in the data.

Power is directly related to type II error.

Power $= 1 - \beta$

There are several ways to increase statistical power. The most common is to increase sample size.

	Reality		
	Drug Works		Drug Does Not Work
Research	Reject	Power	Type I Error
	Not Reject	Type II Error	

SCALE

To convert the world into numbers, we use 4 types of scale: nominal, ordinal, interval, and ratio. Scales of measurement refer to ways in which numbers are categorized.

Note

For the exam, focus on **nominal** and **interval** scales.

Table 2-1. Types of Scale in Statistics

Type of Scale	Description	Key Words	Examples
Nominal (Categorical)	Different groups	This or that	Gender, comparing among treatment interventions
Ordinal	Groups in sequence	Comparative quality, rank order	Olympic medals, class rank in medical school
Interval	Exact differences among groups	Quantity, mean, and standard deviation	Height, weight, blood pressure, drug dosage
Ratio	Interval + true zero point	Zero means zero	Temperature measured in degrees Kelvin

The scales as described below are hierarchically arranged, from **least information** provided (nominal) to **most information** provided (ratio). Any scale can be degraded to a lower scale, e.g., interval data can be treated as ordinal.

- **Nominal scale**
  - Puts people into categories without specifying the relationship between the categories
  - Example is gender, with 2 groups (male and female); other examples include drug versus control group
  - Anytime you can say, “It’s either this or that,” it is nominal scale
- **Ordinal scale** (or rank order)
  - Puts people into categories and specifies the relationship between them (quality)
  - What is not known is *how* different the categories are (quantity)
  - Example is saying Ben is taller than Fred; other examples include class rank in medical school and Olympic medals
- **Interval scale** (or numeric scale)
  - Uses a scale graded in equal increments
  - Allows us to say not only that 2 things are different, but also by *how much*
  - If a measurement has a mean and an SD, treat it as an interval scale
  - Example is the scale of length: we know that 1 inch is equal to any other inch
- **Ratio scale (best measure)**
  - Orders things and contains equal intervals, but also has a **true zero point**
  - Zero is a floor, i.e., you can’t go any lower
  - Example is measuring temperature using Kelvin scale

STATISTICAL TESTS

Selecting the correct statistical test for a research project will depend on the nature of the variables being studied.

Table 2-2. Types of Scale and Basic Statistical Tests

	Variables		
Name of Statistical Test	**Interval**	**Nominal**	**Comment**
Pearson correlation	2	0	Is there a linear relationship?
Chi-square	0	2	Any # of groups
t-test	1	1	2 groups only
One-way ANOVA	1	1	2 or more groups
Matched pairs *t*-test	1	1	2 groups, linked data pairs, before and after
Repeated measures ANOVA	1	1	More than 2 groups, linked data

ANOVA = Analysis of Variance

Correlation (*r*, –1.0 to +1.0)

Correlation, by itself, does not mean causation. A correlation coefficient indicates the degree to which 2 measures are related, not *why* they are related. In other words, it does not mean that one variable necessarily causes the other.

There are 2 types of correlation:

- **Pearson correlation**: compares 2 interval level variables
- **Spearman correlation**: compares 2 ordinal level variables

A **positive value** means that **2 variables go together in the same direction,** e.g., MCAT scores have a positive correlation with medical school grades.

A **negative value** means that the **presence of one variable is associated with the absence of another variable,** e.g., there is a negative correlation between age and quickness of reflexes.

The further from zero, the stronger the relationship ($r = 0$). A **zero correlation** means that **2 variables have no linear relation to one another,** e.g., height and success in medical school.

Correlation can be graphed using a scatterplot, which shows points that approximate a line.

Note

Remember, your **default choices** are:

- Correlation for interval data
- *Chi*-square for nominal data
- *t*-test for a combination of nominal and interval data

Note

On the exam you will not be asked to compute statistical tests, but do recognize how and when they should be used.

You should, however, be able to interpret **scatterplots of data:** positive slope, negative slope, and which of a set of scatterplots indicates a stronger correlation.

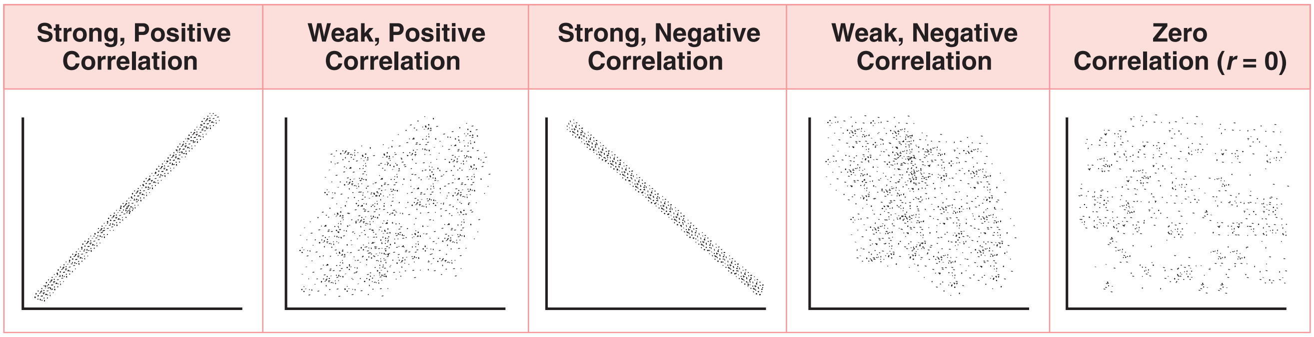


Figure 2-9. Scatterplots and Correlations

t-test

A *t*-test **compares the means of 2 groups** from a single nominal variable, using means from an interval variable to see whether the groups are different. The output of a *t*-test is a "*t*" statistic. It is used for 2 groups only, i.e., compares 2 means. For example, do patients with MI who are in psychotherapy have a reduced length of convalescence compared with those who are not in therapy?

- **Pooled** *t*-test is a regular *t*-test, assuming the variances of the 2 groups are the same
- **Matched pairs** *t*-test involves matching each person in one group with a person in a second group; applies to before-and-after measures and linked data

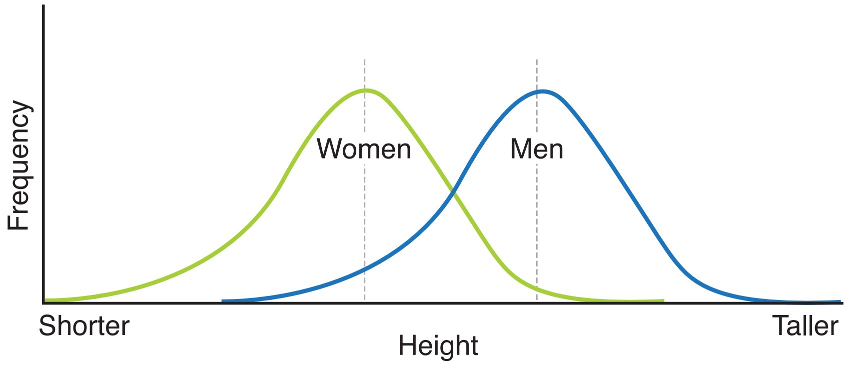


Figure 2-10. Comparison of the Distributions of 2 Groups

Analysis of Variance (ANOVA)

Output from an ANOVA is ≥1 F-statistics.

- **One-way ANOVA** compares means of many groups (≥2) of a single nominal variable using an interval variable. A significant *p*-value means that at least 2 of the tested groups are different.
- **Two-way ANOVA** compares means of groups generated by 2 nominal variables using an interval variable. It can test the effects of several variables at the same time.
- **Repeated measures ANOVA** features multiple measurements of the same people over time.

Chi-square

A *chi*-square tests to see whether 2 nominal variables are independent, i.e., in order to test the efficacy of a new drug, compare the number of recovered patients given the drug with those who were not. *Chi*-square features nominal data only, and any number of groups (2 × 2, 2 × 3, 3 × 3, etc.).

Table 2-3. *Chi*-Square Analysis for Nominal Data

	New Drug	Placebo	Totals
Recovered	45	35	80
Not Recovered	15	25	40
Totals	60	60	120

Review Questions

9. A recent study finds a higher incidence of SIDS for children of mothers who smoke. If the rate for smoking mothers is 230/100,000 and the rate for nonsmoking mothers is 71/100,000, what is the relative risk for children of mothers who smoke?

    A. 159
    B. 32
    C. 230
    D. 3.2
    E. 8.4

10. A researcher wishing to demonstrate the efficacy of a new treatment for hypertension compares the effects of the new treatment versus a placebo. This study provides a test of the null hypothesis that the new treatment has no effect on hypertension. In this case, the null hypothesis should be considered as

    A. positive proof that the stated premise is correct.
    B. the assertion of a statistically significant relationship.
    C. the assumption that the study design is adequate.
    D. the probability that the relationship being studied is the result of random factors.
    E. the result the experimenter hopes to achieve.

11. A standardized test was used to assess the level of depression in a group of patients on a cardiac care unit. The results yielded a mean of 14.60 with confidence interval of 14.55 and 14.65. This presented confidence interval is

    A. less precise, but has a higher confidence than 14.20 and 15.00.
    B. more precise, but has a lower confidence than 14.20 and 15.00.
    C. less precise, but has a lower confidence than 14.20 and 15.00.
    D. more precise, but has a higher confidence than 14.20 and 15.00.
    E. indeterminate, because the degree of confidence is not specified.

12. A recently published report explored the relationship between height and subjects' self-reported cholesterol levels in a sample of 44- to 65-year-old males. The report included a correlation of +0.02, computed for the relationship between height and cholesterol level. One of the possible interpretations of this correlation is:

    A. The statistic proves that there is no definable relationship between the two specified variables.
    B. There is a limited causal relationship between the two specified variables.
    C. A real-life relationship may exist, but the measurement error is too large.
    D. A scatterplot of the data will show a clear linear slope.
    E. The correlation is significant at the 0.02 level.

Questions 13–15

The Collaborative Depression study examined several factors impacting the detection and treatment of depression. One primary focus was to develop a biochemical test for diagnosing depression. For this research, a subpopulation of 300 persons was selected and subjected to the dexamethasone suppression test (DST). The results of the study are as follows:

	Actual Depression	
	NO	YES
DST Results		
Depressed	87	102
Nondepressed	63	48

13. Which of these ratios measures specificity?

    A. 102:150
    B. 102:189
    C. 63:150
    D. 87:150
    E. 63:111

14. Which of these ratios measures positive predictive value?

    A. 102:150
    B. 102:189
    C. 63:150
    D. 87:150
    E. 63:111

15. Which of these ratios measures sensitivity?

    A. 102:150
    B. 102:189
    C. 63:150
    D. 87:150
    E. 63:111

16. Initial research supported a conclusion that a positive relationship exists between coffee consumption and heart disease. However, subsequent, more extensive research suggests that the initial conclusion was the result of a type I error. In this context, a type I error

    A. means there is no real-life significance, but statistical significance is found.
    B. suggests that the researcher has probably selected the wrong statistical test.
    C. results from a nonexclusionary clause in the null hypothesis.
    D. indicates that the study failed to detect an effect statistically, when one is present in the population.
    E. has a probability in direct proportion to the size of the test statistic.

17. A survey of a popular seaside community (population 1,225) found the local inhabitants to have unusually elevated blood pressure. In this survey, just over 95% of the population had systolics between 110 and 190. Assuming a normal distribution for these assessed blood pressures, the standard deviation for systolic blood pressure in this seaside community is most likely

    A. 10
    B. 20
    C. 30
    D. 40
    E. 50

18. A report of a clinical trial of a new drug for herpes simplex II versus a placebo noted that the new drug gave a higher proportion of success than the placebo. The report ended with the statement: *chi*-square = 4.72, $p < 0.05$. In light of this information, we may conclude that

    A. fewer than 1 in 20 will fail to benefit from the drug.
    B. the chance that an individual patient will fail to benefit is less than 0.05.
    C. if the drug were effective, the probability of the reported finding is less than 1 in 20.
    D. if the drug were ineffective, the probability of the reported finding is less than 0.05.
    E. the null hypothesis is false.

19. A recent study was conducted to assess the intelligence of students enrolled in an alternative high school program. The results showed the IQs of the students distributed according to the normal curve, with a mean of 115 and a standard deviation of 10. Based on this information it is most reasonable to conclude that

A. 50% of the students will have an IQ below the standard mean of 100.

B. 5% of the students will have IQs below 105.

C. students with IQs of 125 are at the 84th percentile.

D. 2.5% of the students will have IQs greater than 125.

E. all of the students' scores fall between 85 and 135.

20. A correlation of +0.56 is found between alcohol consumption and systolic blood pressure in men. This correlation is significant at the 0.001 level. From this information we may conclude that:

A. There is no association between alcohol consumption and systolic pressure.

B. Men who consume less alcohol are at lower risk for increased systolic pressure.

C. Men who consume less alcohol are at higher risk for increased systolic pressure.

D. High alcohol consumption can cause increased systolic pressure in men.

E. High systolic pressure can cause increased alcohol consumption in men.

Questions 21-23

To assess the effects of air pollution on health, a random sample of 250 residents of Denver, Colorado, were given thorough checkups every 2 years. This same procedure was followed on a matched sample of persons living in Fort Collins, Colorado, a smaller town located about 60 miles north. Some of the results, presented as percent mortality, are displayed in the table below.

Cumulative Mortality in 2 Communities Over 10 Years

	1975	1977	1979	1981	1983	1985
Denver	4%	6%	10%	15%	22%	28%
Fort Collins	2%	3%	7%	10%	12%	14%

21. This type of study can be best characterized as a

A. cross-sectional study

B. clinical case trial design

C. cross-over study

D. cohort study

E. case-control study

22. According to the data presented in the table, the cumulative relative risk for living in Denver by the year 1981 was

    A. 0.67
    B. 5%
    C. 1.5
    D. 2.0
    E. 1.33

23. What statistical test would you run to test whether there was a difference between the cumulative mortality rate for Denver and Fort Collins in 1985?

    A. *t*-test
    B. ANOVA
    C. Regression
    D. Correlation coefficient
    E. *Chi*-square

24. A study is conducted to examine the relationship between myocardial infarction and time spent driving when commuting to and from work. One hundred married males who had suffered infarcts were selected and their average commuting time ascertained from either the subject, or if the infarct had been fatal, their spouse. A comparison group of 100 married males who had not suffered infarcts was also selected and their average commuting time recorded. When examining this data for a possibly causal relationship between commuting time and the occurrence of myocardial infarcts, the most likely measure of association is

    A. odds ratio.
    B. relative risk.
    C. incidence rate.
    D. attributable risk.
    E. correlation coefficient.

25. A particular association determines membership based on members' IQ scores. Only those people who have documented IQ scores at least 2 SDs above the mean on the Wechsler Adult Intelligence Scale, Revised (WAIS-R), are eligible for admission. Out of a group of 400 people randomly selected from the population at large, how many would be eligible for membership in this society?

    A. 2
    B. 4
    C. 6
    D. 8
    E. 10

26. A physician wishes to study whether moderate alcohol consumption is associated with heart disease. If, in reality, moderate alcohol consumption leads to a relative risk of heart disease of 0.60, the physician wants to have a 95% chance of detecting an effect this large in the planned study. This statement is an illustration of specifying

    A. alpha error.
    B. beta error.
    C. a null hypothesis.
    D. criterion odds.
    E. statistical power.

27. Public health officials were examining a suspicious outbreak of diarrhea in an inner city community child-care center. Center workers identified children with diarrhea and all children at the center were studied. Officials discovered that children who drank liquids from a bottle were more likely to have diarrhea than children who drank from a glass. They concluded that drinking from unclean bottles was the cause of the outbreak. The use of bottles was subsequently banned from the center. The outbreak subsided. Which of the following is the most likely source of bias in this study?

    A. Recall bias
    B. Lead-time bias
    C. Measurement bias
    D. Confounding
    E. Random differences as to the identification of diarrhea

28. Suicides in teenagers in a small Wisconsin town had been a rare event before 11 cases were recorded in 1994. This unusual occurrence led to the initiation of an investigation to try to determine the reason for this upsurge. The researchers suspect that the suicides are linked to the increasing numbers of new families who have recently moved to the town. The best type of study to explore this possibility would likely be a

    A. cohort study.
    B. case-control study.
    C. cross-over study.
    D. cross-sectional study.
    E. community trial study.

Questions 29–38

Which statistical test will most likely be used to analyze the data?

29. Comparing the blood sugar levels of husbands and wives

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

30. Comparing the number of staff who do or do not call in sick for each of 3 different nursing shifts

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

31. Relationship between time spent on studying and test score

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

32. A researcher believes that boys with same-sex siblings are more likely to have higher testosterone levels.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

33. A physician believes that drawing blood is faster with a vacutainer for someone once that person is trained, but faster with a standard syringe for someone with no training.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

34. Twenty rats are coated with margarine and 20 with butter as part of a study to explore the carcinogenic effects of oleo.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

35. To assess the efficacy of surgical interventions for breast cancer, the quality of life, measured on a 10-point scale, of 30 women who underwent radical mastectomies was compared with 30 women who received radiation treatments and 15 women who refused any medical intervention.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

36. Comparison of passing and failure rates at each of 3 test sites.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

37. Comparison of actual measured test scores for students at each of 3 test sites.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

38. Assessing changes in blood pressure for a group of 30 hypertensives 1 week before and 3 months after beginning a course of antihypertensive medication.

    A. *t*-test
    B. *Chi*-square test
    C. One-way ANOVA
    D. Two-way ANOVA
    E. Pearson correlation
    F. Matched pairs *t*-test

39. In a study of chemical workers, 400 workers with respiratory disease and 150 workers without respiratory disease were selected for examination. The investigators obtained a history of exposure to a particular solvent in both groups of workers. Among workers with the respiratory disease, 250 gave a history of exposure to the solvent, compared to 50 of the workers without respiratory disease. The study design can best be described as a

    A. case-control study.
    B. cohort study.
    C. cross-sectional study.
    D. community trial.
    E. comparative clinical trial.

40. The air quality is assessed in two Midwestern cities, one in which a government program has instituted reducing the amount of carbon monoxide emissions allowed, and one without the government program. The rates of respiratory problems in both cities are recorded over a 5-year period. Given the design of this study, an appropriate one-tailed null hypothesis would be

    A. air quality is related to respiratory problems in both of the cities under study.
    B. air quality is related to respiratory problems in the city with the government program but not in the other city.
    C. no evidence will be found for differences in air quality between the two cities.
    D. the rate of respiratory problems in the city with the government program will not be any lower than that of the other city.
    E. air quality will be inversely related to the rate of respiratory problems in both cities.

Answers and Explanations

9. **Answer: D.** Relative risk means divide, compute the ratio between the 2 groups. [230/71 = 3.2]

10. **Answer: D.** This is a definition question. Null hypothesis is a statement of chance, the opposite of what the researcher hopes to find.

11. **Answer: B.** A smaller interval is more precise but less confident. Precise means *narrower* interval. 95% confidence yields a smaller interval than 99% confidence.

12. **Answer: C.** One reason for a near-zero correlation is that the error of measurement is so large that it obscures an underlying relationship. It shows no linear relationship and does not mean cause. The number given is the coefficient, not the *p*-value.

13. **Answer: C.** True negatives, out of all nondiseased. [TN/(TN + FP)] = [63/(87 + 63)].

14. **Answer: B.** True positives, out of all positives. [TP/(TP + FP)] = [102/(102 + 87)].

15. **Answer: A.** True positives, out of all diseased. [TP/(TP + FN)] = [102/(102 + 48)].

16. **Answer: A.** A type I error means the researcher rejected the null hypothesis but should not have. This means that although statistical significance is found, there is no real-world significance. By reversing the clauses in the answer, the correct answer becomes more apparent. Answer choice (D) is a good definition of a type II error.

17. **Answer: B.** If 95% of cases fall between 110 and 190 and the distribution is symmetrical, then the mean must be 150, and the numbers given are 2 standard deviations above and below the mean. This means that 2 standard deviations must equal 40 and that one standard deviation equals 20.

18. **Answer: D.** The key here is the "*p*-value." Ignore the *chi*-square value. If less than 0.05, this gives the chance of a type I error. Therefore, the probability of the finding if the drug was ineffective.

19. **Answer: C.** This is 1 standard deviation above the mean: [115 + 10]. Below this point are 84% of the cases using the normal curve.

20. **Answer: B.** The given correlation is statistically significant at the 0.001 level and can therefore be interpreted. It is a positive correlation, suggesting that high goes with high and low goes with low. Avoid answers which suggest a causal relationship.

21. **Answer: D.** People in the two communities are followed forward in time, and incidence (mortality) is the outcome.

22. **Answer: C.** The key is to focus only on 1981. Relative risk means divide. [15%/10% = 1.5] or 1.5 times the risk.

23. **Answer: E.** Denver versus Fort Collins is one nominal variable with two groups. Dead versus alive is the second nominal variable. Two nominal variables with $n > 25$ = *chi*-square.

24. **Answer: A.** This is a case-control study (infarcts versus no infarcts). Therefore, use an odds ratio. The data is not incidence data, so relative risk does not apply.

25. **Answer: E.** The IQ is scaled to have a mean of 100 and a standard deviation of 15. What percent of the cases are more than two standard deviations above the mean? (2.5%) Therefore, what is 2.5% of 400? (10)

26. **Answer: E.** Power is the chance of detecting a difference in the study if there really is a difference in the real world. The question tells us what chance the researcher will have of finding a difference.

27. **Answer: D.** Bottle versus glass is confounded with age or maturity. The other options, while possible, are unlikely.

28. **Answer: B.** Select suicide cases and compare with nonsuicides (controls).

29. **Answer: F.** Blood sugar levels are ratio data, treated as interval data. Husbands and wives are nominal, but are linked nonindependent, matched pairs; therefore, matched pairs *t*-test.

30. **Answer: B.** Staff either call in sick or do not (nominal variable) over 3 shifts (nominal variable). Two nominal variables with a 2×3 design, *chi*-square.

31. **Answer: E.**"Is there a relationship?" between 2 interval level variables. Pearson correlation.

32. **Answer: A.** Same sex versus no same sex (nominal variable). Testosterone level is assessed as ratio and treated as interval. Therefore, simple *t*-test.

33. **Answer: D.** Vacutainer versus standard syringe (nominal), training versus no training (nominal), and time (interval). Two nominal and one interval two-way ANOVA.

34. **Answer: B.** Margarine versus butter (nominal), cancer versus no cancer (nominal). Therefore, *chi*-square.

35. **Answer: C.** There are 3 types of treatment: surgery, radiation, and none (nominal variable, 3 groups), quality of life on the given scale (interval). Therefore, one-way ANOVA.

36. **Answer: B.** Passing versus failure (nominal), 3 sites (nominal). Therefore, *chi*-square.

37. **Answer: C.** Three sites (nominal) with actual test scores (interval). Therefore, one-way ANOVA.

38. **Answer: F.** Before and after (nominal, two-groups, matched pairs), and blood pressure (interval). Therefore, matched pairs *t*-test.

39. **Answer: A.** Respiratory (cases) versus nonrespiratory disease (controls), looking at history.

40. **Answer: D.** The correct statement needs to be a one-directional statement of no effect. "Not be any lower than" satisfies this criterion.

PART II

Behavioral Science

Developmental Life Cycle 3

Learning Objectives

- ❑ Demonstrate understanding of stages and milestones of development
- ❑ Answer questions about sexuality and gender identity
- ❑ Demonstrate understanding of aging and issues of death and bereavement

STAGES OF DEVELOPMENT

Development occurs along multiple lines: physical, cognitive, intellectual, and social.

Newborns

Newborns have certain preferences:

- Large, bright objects with lots of contrast
- Moving objects
- Curves vs. lines
- Complex vs. simple designs
- Facial stimuli

Table 3-1. Newborn Reflexes

Reflex	Features	Onset	Extinction	CNS Origin
Moro (startle)	Arms and legs extend when child is startled	Birth	5 months	Brain stem/vestibular nuclei
Grasp	Fingers curl around object placed in hand	Birth	5 months	Brain stem/vestibular nuclei
Rooting	Baby turns face toward direction of touch	Birth	5 months	Brain stem/trigeminal
Babinski	• Not pathological in newborns • Stroking bottom of foot causes the toe to move upward (dorsiflexion) instead of downward (hallux flexion); normal in adults	Birth	1 year	Spinal cord

Note

Stranger anxiety is distress in the presence of unfamiliar people.

- Peaks at age eight months
- Can last until age one year

Separation anxiety is distress following separation from a caretaker.

- Onset at age eight months
- Can last until age two years

Milestones

Skills achieved by a certain age are called **milestones**, which are normative markers at median ages.

Some children develop more slowly and some more quickly, so milestones are only approximate and do not have to occur concomitantly. Thus, a child may match the milestones for cognitive development but show slower growth in the social area.

Table 3-2. Cognitive Development Theories

Age	Erikson	Freud	Piaget
Birth–2 years	Trust vs. mistrust • Develop feeling of trust that their wants will be satisfied • If parent is not attentive, will learn to mistrust	Oral • Mouth is the main site of gratification; manifested by chewing, biting, and sucking	Sensorimotor • Begin to learn through sensory observation • Gain control of motor functions through activity, exploration, and manipulation of the environment • Achieve object permanence
2–4 years	Autonomy vs. shame/doubt • Have sense of mastery over themselves and their drives; can be cooperative or stubborn • Gain a sense of separateness from others	Anal • Anus and surrounding area is main site of gratification; primarily involved in bowel functions and bladder control • If harsh toilet training, may become "anally fixated" (obsessive-compulsive personality disorder)	Preoperational • Use symbols and language more extensively • Are egocentric, use animistic thinking, and have a sense of imminent justice • See death as reversible • Lack the law of conservation
4–6 years	Initiative vs. guilt • Initiate both motor and intellectual activity • Start to become sexually curious • Start to develop sibling rivalry	Phallic • Genital area is main site of gratification • Penis envy and fear of castration are evident • Increase in genital masturbation with fantasies involving opposite-sex parent ("Oedipal complex")	

Age	Erikson	Freud	Piaget
6–12 years	Industry vs. inferiority • Enter programs of learning; able to work and acquire adult skills • Learn they are able to master and complete a task	Latency • Formation of the superego; resolution of the Oedipal complex • Sexual interests during this period are believed to be quiescent • Sublimation of sexual energy into energetic learning and play activities	Concrete operational • Replace egocentricity with operational thought, thus can see things through others' perspectives • See death as irreversible (age 10) • Have the law of conservation
Teenage years	Identity vs. role confusion • Develop group identity • Develop preoccupation with appearances • Begin to deal with morality and ethics • Experience "identity crisis" at end of this stage (which Piaget called normative)	Genital • Capacity for true intimacy	• Formal operational abstract thinking acquired
Early adulthood	Intimacy vs. isolation • Experience intimacy of sexual relations and friendships (all deep associations are present) • Develop an ability to care and share with others without fear of losing self		
Middle adulthood	Generativity vs. stagnation • Have and raise children, as well as other interests outside the home • If have no children, develop sense of altruism and creativity		
Late adulthood	Integrity vs. despair • Experience sense of satisfaction with one's life; allows for an acceptance of one's place in life cycle		

SEXUALITY

Gender identity is a child's sense of maleness or femaleness. It is established by age 3. **Sexual identity** is determined by secondary sexual characteristics.

- **Gender dysphoria** is a "disconnect" between gender identity and sexual identity. Boys > girls.
- **Gender role** is determined by behaviors exhibited by a child. It can be congruent or incongruent to the child's gender identity (usually congruent).

Sexual orientation is determined by gender identity:

- Homosexuality: same gender identity (can be ego-syntonic or ego-dystonic; when ego-dystonic, is pathological)
- Heterosexuality: opposite gender identity
- Bisexuality: either gender identity
- Asexuality: neither gender identity

Masturbation is normal at all ages and equal in both genders. When it interferes with normal functioning, it is pathological.

Exploring human sexuality is normal, especially during teenage years, even with same sex partners.

Table 3-3. Tanner Stages of Development

	Female	Both	Male
Stage	Breast	Pubic hair	Genitalia
I	Preadolescent	None	Childhood size
II	Breast bud	Sparse, long, straight	Enlargement of scrotum, testes
III	Areolar diameter enlarges	Darker, curling, increased amount	Penis grows in length; testes continue to enlarge
IV	Secondary mound; separation of contours	Coarse, curly, adult type	Penis grows in length/breadth; scrotum darkens, testes enlarge
V	Mature female	Adult, extends to thighs	Adult shape/size

AGING

The human body undergoes significant changes with age that have both medical and psychological implications for your patients. The leading causes of death for patients age >65 include:

- Heart disease
- Malignancy
- Cerebrovascular disease
- Chronic lower respiratory disease

As such, preventive care and primary or secondary prevention becomes crucial to patient health, improved quality of life, and survival.

Some factors can be modified by behavioral change:

- Smoking = smoking cessation
- Poor diet = low sodium diet (CHF), low cholesterol diet (ACS), low sugar (DM)
- Physical inactivity = exercise

Geriatrics is the subspecialty dedicated to the science of providing medical care to the elderly. As a physician, regardless of specialty, you are likely to encounter and treat elderly patients.

Medical

Medical care of the geriatric population includes preventive care, vaccinations, and screening.

- **Preventive care** may include aspirin therapy and lipid management.
- **Vaccinations**: illness is usually associated with higher morbidity and mortality with older patients, so it is important they receive certain vaccinations.
  - Tetanus
  - Diphtheria
  - Pneumococcus
  - Influenza
- **Screening**: the 2 main areas of screening are cancer and abdominal aortic aneurysm. For older patients, the rule of thumb is to evaluate comorbidities, functionality, and life expectancy before making recommendations for screening tests. In general, the survival screening benefit is not seen unless the patient's life expectancy is >5 years.
  - **Cancer** screening: ages for screening are usually standardized:
    - Breast cancer: women age >40
    - Colorectal cancer: men and women age >50
  - **Abdominal aortic aneurysm** screening: men age 65–75

Psychiatric

- **Depression screening**
  - Age >65 is a risk factor for suicide.
  - Screening appropriate especially when patients have a terminal or debilitating illness.
- **Adjustment disorder**
  - Many life changes can be stressors that require coping mechanisms.
  - Some life changes (e.g., retirement, even when voluntary; illness, etc.) can cause an adjustment disorder.

Physiological

On the exam, you will be expected to recognize physiological changes that are not pathological, but rather due to aging.

- **Sexuality**
  - Sexual interest and activity does not decline significantly with aging
  - Best predictor of sexual activity in the elderly is availability of a partner
  - Changes in men: slower erection, longer refractory period, more stimulation needed
  - Changes in women: vaginal dryness and thinning
- **Sleep**
  - Early morning wakefulness
  - Less deep sleep
  - REM sleep does not significantly decrease until age >85

Financial

Several factors contribute to financial instability in the elderly:

- Inadequate fixed income
  - Social Security (government-provided earned benefit): eligible adults who have worked >40 quarters; dependents of eligible adult (typically the spouse who was a homemaker)
  - Pensions (employer-provided earned benefits)
  - Investment income
- High medical costs
- Low financial literacy: elderly can be exploited by unscrupulous investment advisors and sometimes family members

End-of-Life Care

Talking about life expectancy and end-of-life treatment and expectations is important.

- Patients should be asked about DNR status.
- Patients may have a living will or assign a health power of attorney in the event they can no longer make decisions themselves.
- You have an obligation to tell the patient everything.
- Do not give false hope to patients, but recognize that they might hope for things other than a cure: quality of life, less pain, a painless death.
- Allow patients to talk about their feelings.
- Encourage patients to avoid social isolation and stay engaged in different activities.

Patients may cycle through the Kübler-Ross stages of adjustment. The stages need not occur in order.

- Denial
- Anger
- Bargaining
- Depression
- Acceptance

Hospice care is care for terminally-ill patients with a life expectancy ≤6 months. It provides care and support for patients (and their families) with advanced disease; the goal is to help dying patients with peace, comfort, and dignity.

Hospice care consists of medical care, psychological support, and spiritual support. It may be delivered at specialized facilities or at home.

In the United States, payment for hospice care varies:

- Medicare hospice benefit
- Medicaid hospice benefit
- Private insurance

DEATH AND BEREAVEMENT

Attachment and Loss in Children

According to Bowlby's theory of attachment, children are predisposed at birth to form attachments with others. Over the first two years of life, they form attachments with their primary caregiver.

Separation from a child can lead to the following:

- Protest (usually seen during short-term separation, e.g., up to two weeks)
  - Crying, screaming, and clinging when parents leave
  - Anger toward parent upon return
- Despair
  - Protesting stops
  - Despondency and sadness
  - Child appears calmer but may be withdrawn and disinterested
- Detachment
  - If separation continues, the child will start to engage with others but will reject caregiver and remain angry
  - Indifference upon caregiver's return

Mourning and Loss in Adults

Adults who are bereaved or are mourning the loss of a loved one also go through a period of adjustment. People move back and forth through the stages of adjustment (Kübler-Ross).

Not everyone passes through all stages or reaches adequate adjustment.

Table 3-4. Normal Grief vs. Depression

Normal Grief	Depression
Normal up to 1 year	After 1 year, sooner if symptoms severe
Crying, decreased libido, weight loss, insomnia	Same but more severe
Longing, wish to see loved one, may think they hear or see loved one in a crowd (illusion)	Abnormal overidentification, personality change
Loss of other	Loss of self
Suicidal ideation is rare	Suicidal ideation is common
Self-limited, usually <6 months	Symptoms do not stop (may persist for years)
Antidepressants not helpful	Antidepressants helpful

SUICIDE

Suicide is the 10th leading cause of death in the United States. Men > women; however, women attempt suicide more often (pills/poison).

- Elderly are most successful and attempt less frequently.
- Adolescents attempt more frequently.
- Ethnic group with the highest suicide rate is Native Americans; within this group adolescents > elderly.
- Firearms account for >50% of all suicides.
- 50% have seen a physician in the past month.

High risk factors for suicide include:

- Previous suicide attempt
- Age
- Gender
- High socioeconomic status (SES)
- Unemployed
- Medical/psychiatric comorbidities
- Hopelessness
- Isolation
- Initiation of antidepressant pharmacotherapy (suicide window)

Note

Decreased levels of 5-HIAA (serotonin metabolite) are associated with aggression and suicide.

Theories of Learning and Behavioral Modification

4

Learning Objectives

- ❏ Demonstrate understanding of theories of learning and how different reinforcers are applied
- ❏ Answer questions about behavioral modification, including classical and operant conditioning
- ❏ Answer questions about behavioral models of depression

LEARNING

Learning results from a permanent change in behavior not due to fatigue, drugs, or maturation. There are two main types of learning: classical and operant.

Classical Conditioning

In classical conditioning, a neutral stimulus is associated with an event that usually elicits an unconditioned response. The conditioned response is elicited by the conditioned stimulus after repeated pairings of the unconditioned stimulus (UCS) and conditioned stimulus (CS).

The classic example is the Pavlovian experiment, which pairs the ringing of a bell with the bringing of food. Eventually the sound of the bell elicits the salivary response that previously occurred only with the sight of the food.

Another example is when a patient receives chemotherapy (UCS), which induces nausea (UCR). Eventually, the sights and sounds of the hospital alone (CS) elicit nausea, now a conditioned response (CR).

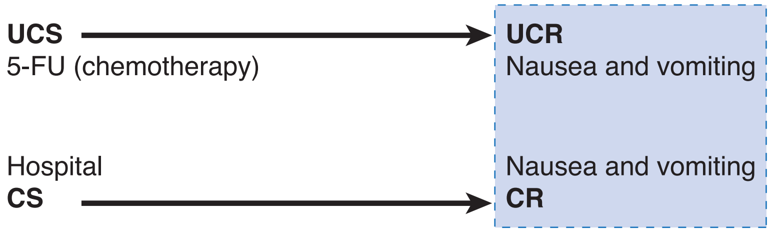


Figure 4-1. Classic Conditioning

Operant Conditioning

In operant conditioning (experiment by B. F. Skinner), learning occurs when a behavior is followed by an event. In the experiment:

- A rat presses a lever to get a pellet of food. (The behavior is called operant because it operates on the environment.)
- After receiving the food, the rat becomes more likely to press the lever because the food is a reinforcing event.
- The role of the reinforcer is to increase the likelihood of a response.

A **primary reinforcer** is the key motivator for behavior. It is often a physiological or psychological necessity, e.g., food, water, and sex.

A **secondary reinforcer** is a stimulus or situation that has acquired its function as a reinforcer after pairing with a stimulus that functions as a reinforcer. Examples often include tokens and money.

Behavior	→	**Reinforcement**	→	**Response**
Drug experimentation		Pleasure		Drug addiction

Types of Reinforcers

There are two types of reinforcers that both increase the probability of a response. Typically, positive reinforcers add a desirable stimulus and negative reinforcers remove an aversive stimulus. No stimulus is universal.

- A **positive reinforcer** is a stimulus that, when applied following an operant response, strengthens the probability of that response occurring.
  - A woman gets a bonus at work after completing a big project; that will make her happy and more likely to perform well again.
- A **negative reinforcer** is a stimulus that, when removed following an operant response, strengthens the probability of that response occurring.
  - A child cleans up his room (response/desired behavior) in order to stop his mother's nagging (negative reinforcer).

Behavioral response to the same stimulus can be different (increased or decreased) from person to person. Do not rely on subjective evaluations of whether the stimulus is unpleasant. An introvert might find a party aversive, while an extrovert would not.

Punishment is a stimulus that will decrease the probability of the response. It usually uses an aversive stimulus to the individual. In punishment, you want to decrease the response.

- A man drives over the speed limit and gets a speeding ticket. The goal of the ticket is for the man to reduce his driving speed.

Extinction refers to the disappearance of a response when it is no longer being reinforced.

Learning theory	Extinction occurs when you	Effect
Classic conditioning	Unpair the unconditioned stimulus (food) with the conditioned stimulus (bell)	Dog does not salivate when bell is rung.
Operant conditioning	Remove reinforcer (food)	Rat stops pressing the lever looking for food.

Types of Reinforcement

In **continuous reinforcement**, every response is followed by a reinforcement. This results in fast learning (acquisition) and fast extinction when reinforcement is stopped.

In **intermittent (or partial) reinforcement**, not every response is reinforced. Learning is slower and response is harder to extinguish.

Suppose a child often throws tantrums, and in the hope that he will stop, the parents ignore him for long periods of time. They don't want to reinforce such behavior with attention. However, if their patience eventually wears thin and they attend to him, they are putting the child on an intermittent reinforcement schedule, which will make it harder to extinguish the tantrums.

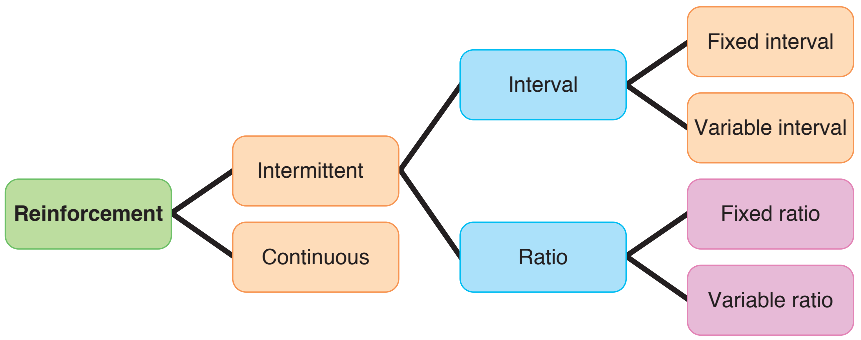


Figure 4-2. Reinforcement

Reinforcement Schedules

Interval schedules are based on the passage of time before reinforcement is given.

- A **fixed interval schedule** reinforces the response that occurs after a fixed period of time elapses. Responses are slow in the beginning of the interval and faster immediately prior to reinforcement (end-of-year bonus).
- A **variable interval schedule** delivers reinforcement after unpredictable time periods elapse (surprise bonus you can get anytime).

Ratio schedules are based on the number of behaviors elicited before reinforcement is given.

- A **fixed ratio schedule** delivers reinforcement after a fixed number of responses. It produces a high response rate (getting a bonus after every three projects completed).
- A **variable ratio schedule** delivers reinforcement after a changing number of responses. It produces the greatest resistance to extinction (getting a bonus after completing undisclosed number of projects).

Table 4-1. Reinforcement Schedules

Interval Schedule	Examples
Fixed interval	• Weekly paycheck • Bonus during holiday season • Gift with each purchase • Weekly quiz
Variable interval	• Surprise bonus • Pop quiz • Listening to radio for favorite song
Ratio Schedule	**Examples**
Fixed ratio	• Piecemeal work • Free sandwich after 10 sandwiches bought • $5,000 to a salesman after each sale of 5 automobiles
Variable ratio	• Slot machines • Door-to-door salesman • Unknown sales bonus

Modeling

In modeling, learning occurs through observation. Watching someone else get reinforcement is enough to change behavior.

BEHAVIORAL MODIFICATION

Classical Conditioning

Systematic desensitization usually begins with imagining oneself in a progression of fearful situations and using relaxation strategies that compete with anxiety. It is often used to treat anxiety and phobias, and is based on the concept of counterconditioning.

- Patients start by creating a list of fear-eliciting stimuli from least stressful to most stressful.
- They then pair their fear-eliciting stimulus with behaviors that elicit unconditioned responses (relaxation).
- When they are relaxed in the presence of the feared stimulus, the fear response disappears.

Exposure is treatment by forced exposure to the feared object; maintained until fear response is extinguished. If you are afraid of heights, you will ride up the elevator until the fear ceases.

Flooding, or massive exposure, is where patients are exposed to a maximum intensity anxiety-producing situation. If imagined, it is called implosion. If you are afraid of bugs, you will be locked in a room with millions of bugs.

Aversive conditioning occurs when a stimulus that produces undesired behavior is paired with an aversive stimulus. In treatment of alcoholism, patients are given disulfiram, which makes them sick when they drink alcohol.

Operant Conditioning

Shaping (or successive approximations) achieves final target behavior by reinforcing successive approximations of the desired response. Reinforcement is gradually modified to move behaviors from the more general to the specific responses desired. A boy with autism is rewarded when he utters one word and subsequently has to utter more words to obtain the same reward.

Stimulus control is where a stimulus inadvertently acquires control over behavior. When this is true, removal of that stimulus can extinguish the response. Watching TV while eating will increase weight, so in order to lose weight you must stop watching TV.

Biofeedback (neurofeedback) uses external feedback via instruments to provide usually unperceived biological information subsequently used to modify internal physiologic states. Certain functions of the autonomic nervous system (pulse, blood pressure, muscle tone, pain perception) can be manipulated through the technique of biofeedback.

Fading is gradually removing the reinforcement without the individual becoming aware of the difference.

- Patients receive pain medication after surgery, but each dose is smaller until discontinuation.
- Nicotine patch begins with 21 mg and is later reduced to 14 mg and then 7 mg.
- Patients are unaware during this process that they are receiving less nicotine.

Behavioral Models of Depression

Learned helplessness (or the animal model of depression) is where all normal avoidance responses are extinguished. If a rat is shocked and not allowed to escape, eventually the rat will not take an obvious avoidance route even when it is offered.

Symptoms of helplessness in animals include passivity, norepinephrine depletion, and difficulty learning responses that produce relief and weight/appetite loss.

- Characterized in people by an attitude of "when nothing works, why bother." A woman in an abusive relationship who perceives she cannot escape the abuse will give up and become depressed.
- Increased levels of GABA in hippocampus decrease the likelihood of learned helplessness response.

Low rate of response-contingent reinforcement is another explanation for depression. The person receives too little predictable positive reinforcement and may lack the social skills necessary to elicit this positive reinforcement. Depression can be seen as a prolonged extinction schedule; it results in passivity.

- A man who feels he receives no positive reinforcement from his spouse can become depressed, even if he seems otherwise successful. A caring and giving father who feels unappreciated by his family might become depressed.

Defense Mechanisms 5

Learning Objectives

- ❑ Define the components of psychic structures
- ❑ Describe how the different defenses are used to manage internal conflict

PSYCHIC STRUCTURES

Psychic structures are based on Freudian theory.

The **id** controls primitive instincts and drives (what we want to do):

- Present at birth
- Influences sex and aggression

The **ego** tries to "accommodate" reality:

- Rational
- Resolves conflicts between id and superego

The **superego** determines our conscience or moral compass (what we ought to do):

- Begins development by age 5
- Learned from caretakers
- Insists on socially acceptable behavior, sometimes to the point of individual deprivation
- Can be punitive

DEFENSE MECHANISMS

Defenses are the primary tools of the ego used to manage the internal conflicts between the id and superego. They are the means by which the ego wards off anxiety, and controls instinctive urges and unpleasant effects (emotions).

- All defenses are unconscious, with one exception: suppression.
- Defenses change over time; we are only aware of our defenses in retrospect.
- Defenses are adaptive as well as maladaptive.

Narcissistic Defenses

Projection is when a person attributes his own wishes, desires, thoughts, or emotions to someone else. Internal states are perceived as a part of someone else or of the world in general.

- A cheating spouse accuses partner of cheating.
- A girl talks about her doll as having certain feelings, which are really what the girl feels.

This is the main defense mechanism seen in paranoid personality disorder. Paranoia results from the use of projection.

Denial is not allowing reality to penetrate to avoid acknowledgment of a painful aspect of reality.

- After surviving a heart attack, a patient insists on continuing his lifestyle as if nothing had happened.
- A woman prepares dinner for her husband expecting him to come home, even though he died a month earlier.
- Substance users are often "in denial," claiming that they are not addicted and do not have a problem in the face of clearly dysfunctional or dangerous behavior.

Denial is often the first response to bad news, such as the impending death of a loved one or oneself.

Splitting is when people and things in the world are idealized (all good) or devalued (all bad). The world is pictured in extreme terms rather than a more realistic blend of good and bad qualities.

- "This doctor is a miracle worker, but that doctor is totally incompetent."
- "He's just so perfect and wonderful," says a teenage girl in love.
- "No one from that family will ever amount to anything; they are all just plain no good."

This is the main defense mechanism seen in borderline personality disorder. Prejudice and behavioral stereotypes are also a result of splitting.

Immature Defenses

Blocking is a temporary, or transient, block in thinking or an inability to remember.

- A student is unable to recall the fact needed to answer the exam question, although he recalls it as he walks out of the exam.
- In the middle of a conversation, a woman pauses, looks confused, and asks what she was just talking about.
- In a conversation you forget someone's name.

Blocking often happens in embarrassing moments.

Regression is returning to an earlier stage of development you have already completed (unconscious childish behavior in an adult).

- A husband speaks to his wife in "baby talk" when he is sick.
- A man assumes a fetal position after a traumatic event.
- A previously toilet trained child wets the bed following the birth of a new sibling.

Somatization is when psychological conflict is converted into bodily symptoms.

- A student gets a headache while taking an exam.
- A woman feels queasy and nauseated before asking someone out on a date.
- A man who witnesses a traumatic event becomes blind.

This is the main defense mechanism of somatic symptom disorders.

Introjection (identification) is when we acquire characteristics of others as our own. It is the unconscious form of imitation. Introjection is the opposite of projection.

- A resident dresses and acts like the attending physician.
- A child scolds her friend out loud in the same manner that she was scolded by her mother.
- A teenager adopts the style and mannerisms of a rock star.

This defense mechanism is used in psychotherapy.

Anxiety Defenses

Displacement is when the target of an emotion or drive changes to a substitute target.

- A recently disciplined employee yells at his wife instead of his boss.
- A woman watching a movie featuring love scenes with a handsome actor goes out and seduces an unattractive man.
- In family therapy, one child in a family is often singled out and blamed for all the family's problems, i.e., treated as a scapegoat.

This is the defense mechanism seen in phobias.

Repression is when an idea or feeling is withheld from consciousness. It is also called unconscious forgetting.

- A child who was abused by her mother and treated for the abuse now has no memory of any mistreatment by her mother.
- A man who survived 6 months as a hostage cannot recall anything about his life during that time period.

This is one of the most basic defense mechanisms.

Isolation of affect is the separation of an idea or event from the emotions (affect) that accompany it.

- A child who has been beaten discusses the beatings without any display of emotion.
- A combat pilot is calm while ejecting out of his plummeting aircraft.
- A patient who recently severed his finger in an accident describes the incident to his physician with no emotional reaction.

This is an important adaptive defense mechanism for self-preservation.

Intellectualization is when facts and logic are used to avoid confronting emotions.

- A patient with a bone protruding from his leg focuses on the physics that allow such an event to occur.
- A medical student speaks excessively about medical details in order to avoid the emotional content of a bad diagnosis.
- A boy who, for the first time, is about to ask a girl out talks with his friend about the importance of mating rituals for the long-term survival of the species and the mechanisms by which societies arrange for these rituals.

Physicians who are too concerned with the technical aspects of the profession and not enough with the patient may well be using intellectualization.

Acting out is when an emotional or behavioral outburst masks underlying feelings or ideas.

- A child throws temper tantrum when abandoned
- New-onset drug use in an adolescent boy after parents' divorce
- "Whistling in the dark" to hide underlying fear

This is a defense mechanism that can be seen in borderline and antisocial personality disorders.

Rationalization is when rational explanations are used to justify attitudes, beliefs, or behaviors that are unacceptable. This is not a reasoned action, but a search for reasons to allow an unacceptable action.

- A murderer saying, "Yes, I believe killing is wrong but I killed him because he really deserved it."
- A teenage girl who makes a vow of chastity until marriage tells herself that oral sex is not really sex, and can give a string of reasons.
- An alcoholic man tells his wife that he drinks because of stress at work.

This defense mechanism is seen in substance use disorders.

Reaction formation is when an unacceptable impulse is transformed into its opposite. Excessive overreaction can be a sign of reaction formation.

- A student who always wanted to be a physician expresses relief and says, "This is the best news I've ever heard," after not being accepted into medical school.
- A teenage boy intrigued by "dirty pictures" organizes an anti-pornography campaign.
- Two coworkers fight all the time because they are actually very attracted to each other.

This defense mechanism is commonly seen in obsessive-compulsive disorder and anxiety disorders.

Undoing is performing an act to undo a previous unacceptable act or thought.

- A man who is sexually aroused by a woman he meets immediately leaves and buys his wife flowers.
- Can include superstitions such as throwing salt over your shoulder to avoid bad luck.
- A man repeatedly checks to make sure the burners on the stove are turned off before leaving the house because he is fearful the house will burn down.

This defense mechanism is seen in obsessive-compulsive disorder.

Passive-aggression is when hostility is expressed covertly.

- A patient angry with her physician shows up late for appointments.
- A student agrees to share class notes with classmates but goes home without sharing them after they upset her in class.
- A communications director does not take questions from people who challenge his views.

The feelings of hostility are unconscious, and the person using the defense is generally unaware of them. If you consciously set someone up, it is not a defense, but simply being mean. This defense mechanism is seen in borderline personality disorders and young children.

Dissociation separates the self from one's experience.

- A woman who was raped reports that she felt "as if she was floating on the ceiling" watching it happen.
- The survivor of an automobile accident tells of the feeling that everything happened in slow motion.
- A child who was sexually abused recalls only the "bad man who came to her in her dreams."

This is the primary defense mechanism in dissociative disorders.

Mature Defenses

Humor permits the overt expression of feelings and thoughts without personal discomfort.

- A student smiles when he realizes that a particularly intimidating professor looks like a penguin.
- An overweight comedian makes jokes about being fat.

Laughter covers the pain and anxiety.

Sublimation is when impulse gratification is achieved by channeling the unacceptable or unattainable impulse into a socially acceptable direction.

- Jack the Ripper becomes a surgeon.
- A patient with exhibitionist fantasies becomes a stripper.

Many forms of art and literature spring from sublimation, considered by some to be the most mature defense mechanism.

Suppression is the conscious decision to forget or ignore.

- A student with a pending exam decides to forget about it and go out for the evening.
- A woman who is afraid of heights ignores the drop of a steep cliff to appreciate the beautiful view.
- A terminally-ill cancer patient puts aside his anxiety and enjoys a family gathering.

Suppression is the **only conscious defense mechanism**.

Psychological Health and Testing 6

Learning Objectives

- ❏ Answer questions about stress and how it affects the body
- ❏ Demonstrate understanding of how to calculate intelligence testing
- ❏ Demonstrate understanding of the various types of personality testing

STRESS

Physiologic changes in response to stress include key stress response pathway: hypothalamic-pituitary-adrenal axis.

- Cortisol levels rise, then fall, within 24 hours after stressor.
- Cortisol levels spike again 48–72 hours after stressor.

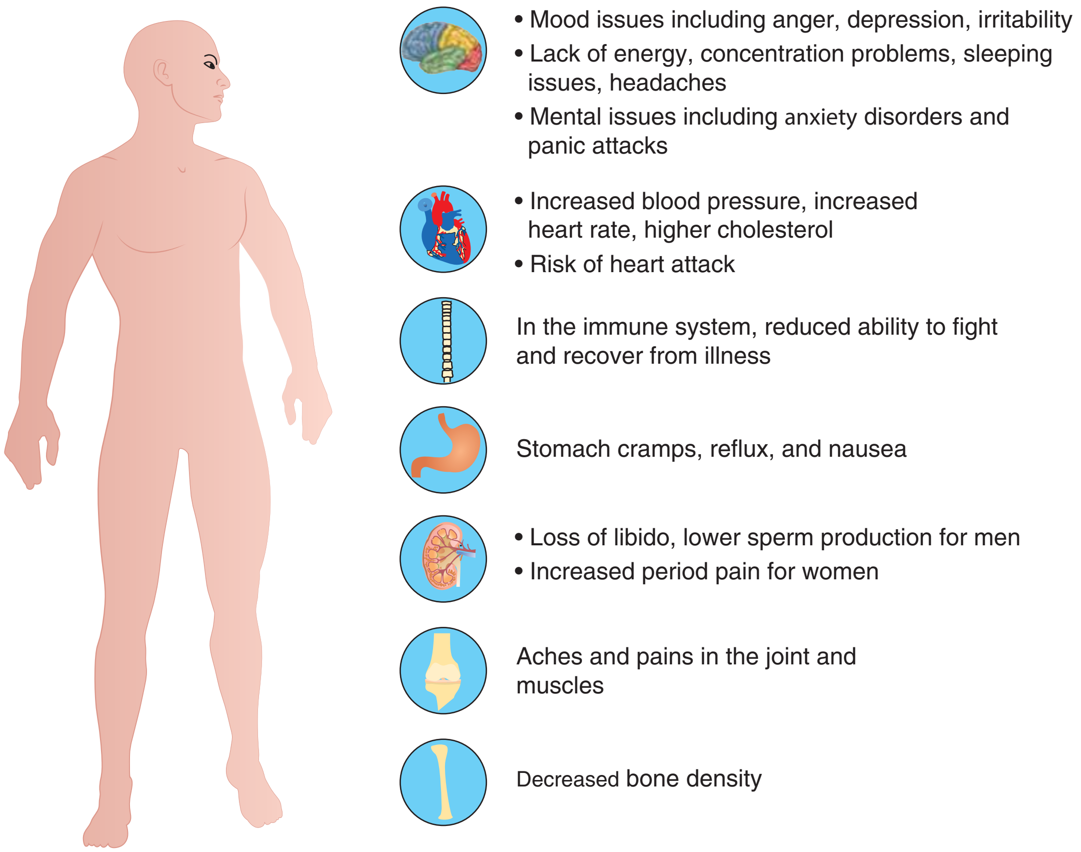


Figure 6-1. Effects of Stress on the Body

Type A and B Personalities

Type A personality is a cluster of behavioral traits that has been associated with increased prevalence and incidence of coronary heart disease.

- Tends to be impatient, competitive, preoccupied with deadlines, and highly involved with work
- Key component of type A behavior: how they handle hostility
- Has increased incidence of coronary heart disease, even after controlling for the major risk factors (systolic blood pressure, cigarette smoking, cholesterol)
- If they survive a first heart attack, less likely than type B to have a second attack

Type B personality lives at lower stress levels. When faced with competition, they do not mind losing.

Stress and Illness

Mentally healthy individuals do not deteriorate in physical health as quickly as do those in poor mental health. Chronic anxiety, depression, and emotional maladjustment predict negative health events later in life.

The Holmes and Rahe scale is used to quantify stressful life events.

- Different life events contribute different weightings to the total score.
- The death of a spouse is weighed as the most stressful event.
- There is a positive correlation between stressful life events and developing illness.

Table 6-1. Holmes and Rahe Life Stress Inventory

Life Event	Mean Value
Death of spouse	100
Divorce	73
Marital separation from mate	65
Detention in jail or other institution	63
Death of a close family member	63
Major personal injury or illness	53
Marriage	50
Being fired at work	47
Marital reconciliation	45
Retirement from work	45
Major change in the health or behavior of a family member	44
Pregnancy	40
Sexual difficulties	39

Gaining a new family member (birth, adoption, older adult moving in, etc.)	39
Major business adjustment	39
Major change in financial state (a lot worse or better than usual)	38
Death of a close friend	37
Changing to a different line of work	36
Major change in number of arguments with spouse (a lot more or less)	35
Taking on a mortgage (for home, business)	31
Foreclosure on a mortgage or loan	30
Major change in responsibilities at work (promotion, demotion)	29
Son or daughter leaving home (marriage, college, military)	29
In-law troubles	29
Outstanding personal achievement	28
Spouse beginning or ceasing work outside the home	26
Beginning or ceasing formal schooling	26
Major change in living conditions (new home, remodeling, deterioration, etc.)	25
Revision of personal habits (dress, associations, quit smoking, etc.)	24
Troubles with the boss	23
Major changes in working hours or conditions	20
Changes in residence	20
Changing to a new school	20
Major change in usual type and/or amount of recreation	19
Major change in church activity (a lot more or less)	19
Major change in social activities (clubs, movies, visiting)	18
Taking on a loan (car, TV, freezer)	17
Major change in sleeping habits (a lot more or less)	16
Major change in number of family get-togethers (a lot more or less)	15
Major change in eating habits (a lot more or less, eating hours, surroundings)	15
Vacation	13
Major holidays	12
Minor violations of the law (traffic ticket, jaywalking)	11

To find your score, add up all your points:

- **<150 points:** relatively low amount of life change and low susceptibility to stress-induced health problems
- **150–300 points**: 50% chance of a major stress-induced health problem in next 2 years
- **>300 points**: odds increase to 80% chance of a major stress-induced health problem in next 2 years

TESTING

Intelligence Testing

Intelligence quotient (IQ) is a general estimate of the functional capacity of a person; 70% is inherited, with recent studies suggesting it is mostly from the mother. IQ is not an absolute score, but a comparison among people. Distribution mean is 100, and standard deviation is 15.

To calculate IQ, use the following:

- **Mental age (MA)** method: IQ = MA/CA (chronological age) × 100
- **Deviation from the norm** method: mean IQ = 100 and SD = 15
    - Intellectual disability <2 SD below the mean

There are several commonly used IQ tests:

- Wechsler Adult Intelligence Scale, Revised (WAIS-R): for adults age ≥17
- Wechsler Intelligence Scale for Children, Revised (WISC-R): for children age 6–17
- Wechsler Preschool and Primary Scale of Intelligence (WPPSI): for children age 4–6
- Stanford-Binet Scale: for children age 2–18

Table 6-2. Distribution of IQ Scores in the General Population

Range	Label	Distribution
<69	Intellectual disability	About 2.5% of population
70–79	Borderline	
80–89	Low average	
90–109	Average	About 50% of population
110–119	High average	
120–129	Superior	
>130	Very superior	About 2.5% of population

Personality Testing

Objective tests have the following features:

- Utilize simple stimuli (usually questions)
- Have a restricted range of possible responses (choose from provided options)
- Scored mechanistically using scoring key
- Require no clinical experience to score

In **criterion-referenced tests**, results are given meaning by comparing them with a preset standard: "Every student who scores above 75% will pass."

In **norm-referenced tests**, results are given meaning by comparing them with a normative group: classic example is Minnesota Multiphasic Personality Inventory (MMPI-2), with >550 T/F questions, a validity scale, and a lie scale.

Projective tests utilize ambiguous stimuli and have a wide range of possible responses. Response meaning is interpreted by clinical correlation between collected cases of responses and personal characteristics (psychopathologies); tests are scored by experienced clinicians using consensual standards.

- **Rorschach inkblot test:** patient is asked to look at an inkblot and report what is seen
- **Thematic apperception test** (TAT): patient is asked to tell a story about what is going on in the picture
- **Sentence completion test:** patient is asked to complete a set of sentence stems with the first thing that comes to mind
- **Projective drawings:** patient is given a sheet of paper and asked to draw a house, a tree, a person, a family, etc. (very useful for children, who are unlikely to be able to realistically complete any other personality test)

Note

Stanford-Binet was the first formal IQ test (1905) for children age <18. Today, it is most useful for children age <6, the impaired, and the very bright.

Note

The MMPI-2 is the most widely used personality test.

Substance Use Disorders 7

Learning Objectives

- ❑ Demonstrate understanding of how alcoholism and tobacco use relate to mortality
- ❑ Demonstrate understanding of how genetics relates to alcoholism and tobacco use
- ❑ Answer questions about the physiology of addiction
- ❑ Answer questions about how substance use disorders can affect pregnancy
- ❑ Demonstrate understanding of how to diagnose and treat a substance use disorder

ALCOHOL AND TOBACCO USE

Alcoholism is the most expensive health problem in the United States, costing >$100 billion/year for alcohol-related illness and death. Tobacco, however, accounts for more loss of life. The best way to reduce long-term mortality is to eliminate smoking.

- Alcohol is most abused drug for all ages; ~10% of all adults are problem drinkers (men > women).
- Alcohol is most widely used illicit drug for teenagers (marijuana is most widely used illicit drug overall).
- Binge drinking is becoming more common; proportion of heavy drinkers age <20 has increased.
- Alcoholism rates are higher for low-SES groups, though they recover sooner.
- Alcohol use has been implicated in 15% of all car accidents, and in 50% of all MVAs not involving a pedestrian, auto accident deaths, homicides (killer or victim), and hospital admissions.

GENETICS

- Concordance rates are higher for monozygotic versus dizygotic twins.
- Twins born to alcoholic parents and raised by non-alcoholic parents are more likely to become alcoholics.

  If biologic father was an alcoholic, the incidence of alcoholism in men adopted into nonalcoholic families is equal to the incidence of alcoholism in sons raised by biologic alcoholic fathers.
- Family history of alcoholism increases likelihood of major depression in offspring.

PHYSIOLOGY

The addiction pathway in the brain is the mesolimbic dopamine pathway. Activation of this pathway accounts for the euphoric feelings of substance abuse which are positively reinforcing and increase the likelihood of subsequent use and potential addiction.

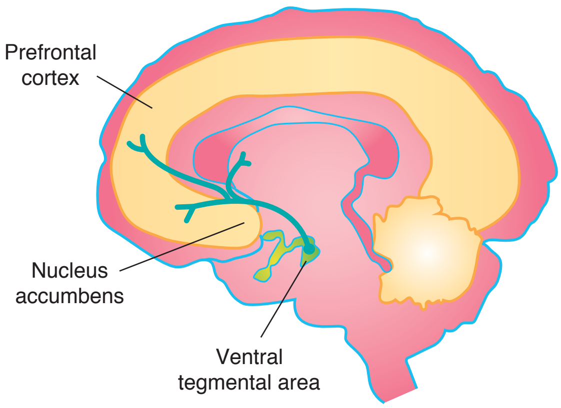


Figure 7-1. Nucleus Accumbens (NAc) Pathway

Stimulus → Cerebral cortex → Ventral tegmental area → Nucleus accumbens

↙ Serotonin (gives body the impression of satisfaction so cravings are reduced)

↘ Dopamine (increased desire for stimulus)

Neurotransmitters involved in the addiction pathway include dopamine, which increases desire for stimulus, and serotonin, which gives the body the impression of satisfaction so cravings are reduced.

PREGNANCY

Women are encouraged to stay away from drugs and alcohol during pregnancy because of complications related to the drug/alcohol use. Drinking between weeks 6–9 (when some women might be unaware they are pregnant) is most likely to lead to facial abnormalities associated with **fetal alcohol syndrome** (FAS). FAS can cause growth problems, mental or behavioral problems, and abnormal facial features.

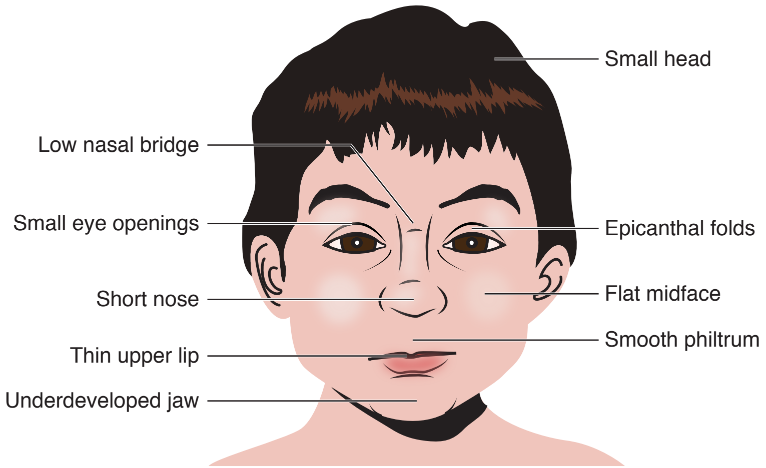


Figure 7-2. Effects of Fetal Alcohol Syndrome

DIAGNOSIS AND MANAGEMENT

Self-Awareness

Most substance users do not believe they have a problem. Denial and rationalization become their main defense.

- Many substance abusers function in society, so it is hard for them to acknowledge they have a problem related to drug and alcohol.
- Typically, something significant (e.g., "hit rock bottom") has to happen to them in order to realize there is a problem.

The CAGE questionnaire is a widely used **screening test** for problem drinking and potential alcoholism.

- Have you ever tried to **C**ut down on alcohol intake and not succeeded?
- Have you ever been **A**nnoyed about criticism concerning your drinking?
- Have you ever felt **G**uilty about your drinking behavior?
- Have you ever had to take a drink as an **E**ye-opener in the morning to relieve the anxiety and shakiness?

There are common stages by which people tend to change their behavior.

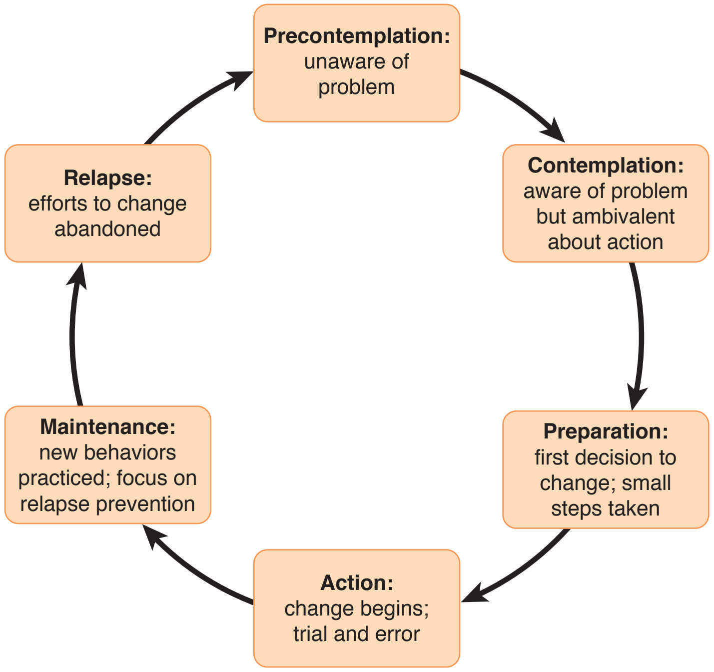


Figure 7-3. Stages of Behavioral Change

The cycle may repeat until sobriety is established.

Alcohol abuse can cause many medical complications: cirrhosis, alcoholic hepatitis, pancreatitis, gastric or duodenal ulcer, esophageal varices, middle-age onset of diabetes, gastrointestinal cancer, hypertension, peripheral neuropathies, myopathies, cardiomyopathy, cerebral vascular accident, erectile dysfunction, vitamin (thiamine) deficiencies, pernicious anemia, and brain disorders including Wernicke-Korsakoff syndrome. Chronic alcohol use can lead to cognitive decline.

Laboratory evidence of alcohol use will include:

- Increased transaminases (AST/ALT) (typically 2:1 ratio)
- Increased bilirubin
- Increased gamma GT (recent alcohol intake)
- Breathalyzer
- Blood alcohol level

Diagnosis

Diagnoses will be of both intoxication with and withdrawal from drugs and alcohol. Oftentimes the diagnosis is clinical. Confirm with urine and blood toxicology screens.

- **Intoxication**: impairment due to acute drug use
- **Withdrawal**: effects of cessation or reduced drug use once patient develops tolerance for the drug (**tolerance** is the diminished efficacy of a drug after repetitive use; requires increased dosage for the same effect)
- **Substance use disorders** may have legal ramifications: driving under the influence (DUI) or driving while intoxicated (DWI)
  - **Use disorders** include the presence of cravings, tolerance, withdrawal, continued use despite adverse consequences, negative impact on home, work and social settings

Treatment

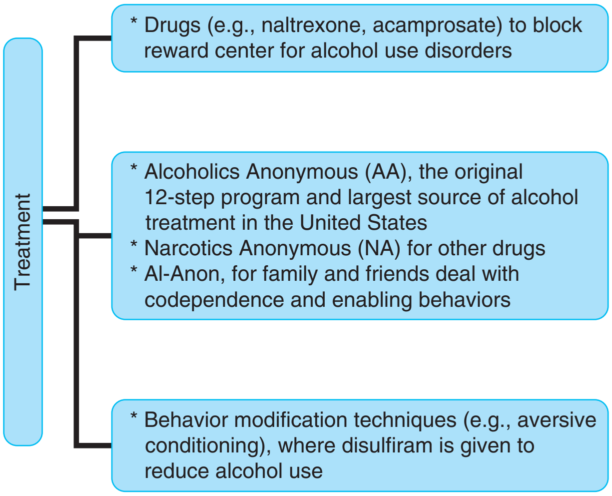


Figure 7-4. Treatment Options for Substance Abuse

Disulfiram may work as an adjunct to psychosocial treatment to reduce alcohol use. Patients must be able committed to maintaining abstinence and to taking the medication.

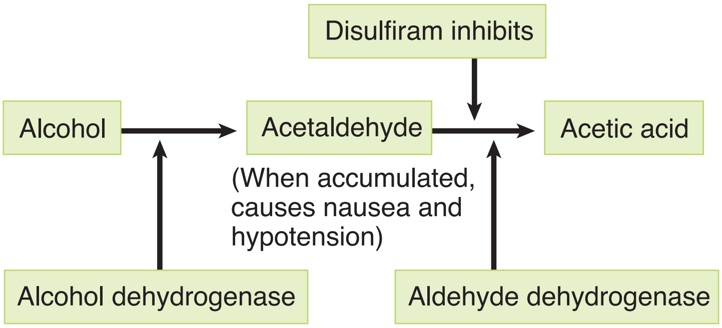


Figure 7-5. Disulfiram Treatment

Note

Alcoholics Anonymous (AA) is considered a spiritual program; it features regular meetings and sponsors who provide substitute dependency, social support, and external reminders that drinking is aversive.

Note

Both naltrexone and acamprosate are effective at reducing cravings, but naltrexone may be used while the patient is still using alcohol.

Table 7-1. Signs and Symptoms of Intoxication/Withdrawal and Treatment

Substance	Signs & Symptoms of Intoxication	Treatment of Intoxication	Signs & Symptoms of Withdrawal	Treatment of Withdrawal	Location/Effect of Drug Action
Alcohol	Talkative, gregarious, moody, disinhibited	If severe, consider mechanical ventilation	Tremors, hallucinations, seizures, DTs	Thiamine, MVI, folic acid, benzodiazepines	Decreases glutamate and increases GABA
Amphetamine *cocaine	Euphoria, hypervigilance, autonomic hyperactivity, weight loss, pupillary dilatation, perceptual disturbances	Antipsychotics and benzodiazepines, antihypertensives	Anxiety, tremors, headaches, increased appetite, depression, risk of suicide	If suicidal, consider antidepressants	Mesolimbic pathway, nucleus accumbens
Bath salts *synthetic cocaine, flakka	• Irritability, perceptual disturbances, agitation • Symptoms can last up to 1 week	Antipsychotics and/or benzodiazepines	Anxiety and depression	If suicidal: consider SSRIs	Not yet known
Cannabis	Impaired motor coordination, slowed sense of time, social withdrawal, increased appetite, conjunctival injection, psychosis	Antipsychotics if needed	Irritability, anxiety	N/A	Inhibitory G protein, GABA, increased serotonin
K2/spice *synthetic marijuana	Perceptual disturbances, agitation, seizures	Antipsychotics and/or benzodiazepines	Not yet known	N/A	Not yet known
Hallucinogens	Ideas of reference, perceptual disturbances, impaired judgment, dissociative symptoms	• Place in quiet room • Antipsychotics, benzodiazepines	N/A	N/A	Stimulate glutamate and serotonin
Inhalants	• Belligerence, apathy, aggression, impaired judgment, stupor or coma • Nasal crusting, rash, drunken appearance, dilated pupils • Use can be fatal • Parkinsonism has been associated with huffing	Antipsychotics	Nausea, excessive sweating, muscle cramps, headaches, chills, agitation, shaking, and hallucinations	Benzodiazepines, antipsychotics	GABA, cerebellum
Opiates	Apathy, dysphoria, pinpoint pupils, drowsiness, slurred speech, coma, death	Naloxone	Fever, chills, runny nose, diarrhea, muscle spasms, cramps	Clonidine, methadone, buprenorphine	• Mu, kappa, and delta receptors • Nucleus accumbens

Substance	Signs & Symptoms of Intoxication	Treatment of Intoxication	Signs & Symptoms of Withdrawal	Treatment of Withdrawal	Location/Effect of Drug Action
Krokodil (desomorphine) *synthetic opiate	Skin, blood vessel, bone, and muscle damage leading to gangrene, phlebitis, sepsis	Symptomatic	Not yet known	N/A	Not yet known
Phencyclidine (PCP)	Belligerence, psychomotor agitation, violence, nystagmus, hypertension, seizures	• Place in quiet room • Antipsychotics • Benzodiazepines	• Elevated body temperature, seizures, and muscle breakdown • Muscle twitching, agitation, and hallucinations may also occur	Benzodiazepines, antipsychotics	Antagonist of N-methyl D-aspartate glutamate receptors
Anabolic steroids	Irritability, aggression, mood instability, psychosis	Antipsychotics	Depression, headaches, anxiety	If depressed, consider SSRIs	May activate dopamine and serotonin release
Ecstasy (MDMA, Molly, E, X)	Euphoria, mild hallucinations, visual distortions, enhanced sensations, hyperthermia, bruxism, autonomic hyperactivity, seizures, dry mouth	Dantrolene, benzodiazepines Hydration	Depression, anxiety, and panic attacks	SSRIs	• Increases serotonin and dopamine • Affects hypothalamus (drinking behavior/body temp); motor neurons of spinal cord (muscle spasms and jaw clenching)
Benzodiazepine	• Slurred speech, confusion, memory deficits, falls • Respiratory depression (rare)	Supportive flumazenil or mechanical ventilation if needed	• Increased anxiety, tremors • Insomnia • Seizures	Benzodiazepines	GABA
Barbiturates	• Restlessness, agitation, insomnia, N/V, anxiety • Tremors, seizures, hallucination, increased heart rate • Respiratory depression	Supportive mechanical ventilation if needed	• Anxiety, depression • Cognitive impairments • Memory deficits, lack of attention • Seizures, delirium	Phenobarbitol	GABA

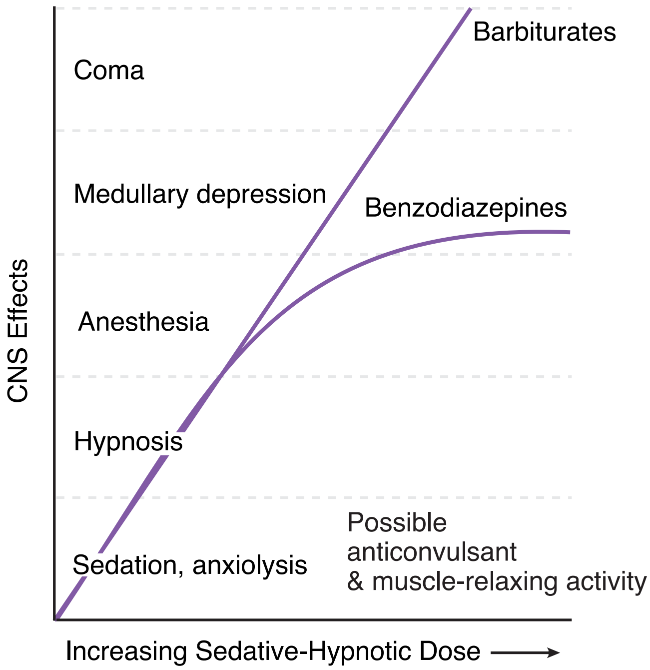


Figure 7-6. CNS Effects

Sleep and Sleep Disorders 8

Learning Objectives

- ❑ Demonstrate understanding of the sleep stages and their associated waves
- ❑ Answer questions about how sleep deprivation can affect the body
- ❑ Demonstrate understand of the different sleep disorders and how to treat

SLEEP ARCHITECTURE

Sleep consists of 2 distinct states: NREM and REM.

Nonrapid Eye Movement (NREM) alternates with REM sleep throughout the sleep period. It is divided into 3 stages on the basis of EEG criteria:

- Slowing of the EEG rhythms
- Higher muscle tone
- Absence of eye movements
- Absence of “thought-like” mental activity

NREM is an idling brain in a movable body.

Rapid Eye Movement (REM) is an awake brain in a paralyzed body:

- Aroused EEG pattern
- Sexual arousal
- Saccadic eye movements
- Dreaming

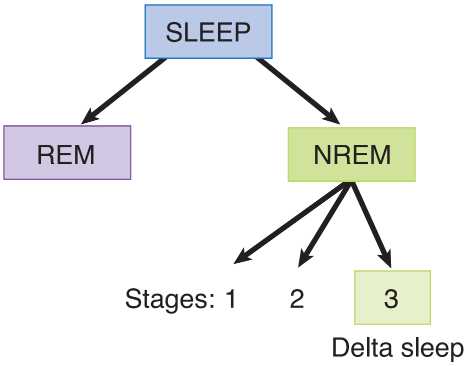


Figure 8-1. Stages of Sleep

Figure 8-2. Sleep Stages and Waves

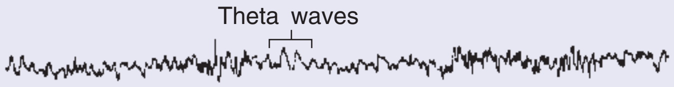
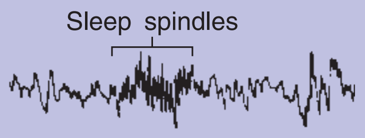
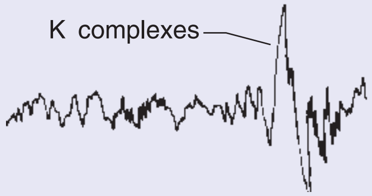
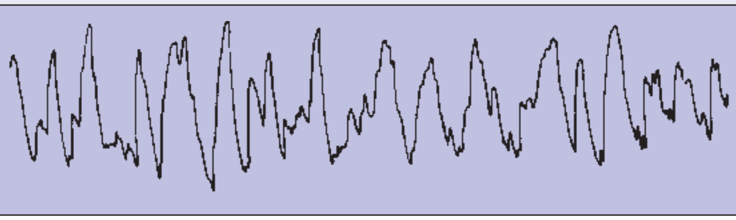
Stage of Sleep	Wave Associated with Stage	EEG Pattern Associated with Waves
1	Theta	
2	Sleep spindles	
2	K complexes	
3	Delta	

Table 8-1. Sleep Facts

Stage 2	Longest stage of sleep
Stage 3	• Deepest stage of sleep; delta sleep is restorative • Tends to decrease in the elderly
Sleep latency	About 5–15 min from time one goes to bed and falls asleep
REM latency	About 90 min from time one falls asleep to first REM period
REM	• First REM period of night is 5–15 min and last one is 20–40 min • REM increases as night goes on • Greater amounts in second half of night • Easiest to arouse • Memories are consolidated by hippocampus
NREM	Greater amounts in first half of night

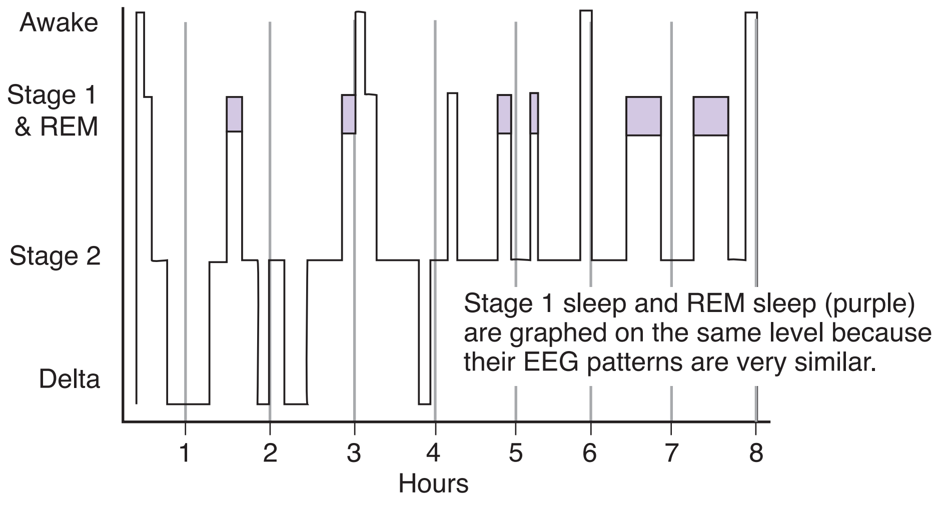


Figure 8-3. Sleep Architecture Diagram

Sleep Deprivation

The cerebral cortex shows the greatest effects of sleep deprivation but has the capacity to cope with one night's sleep loss. In sleep-deprived individuals:

- Cortisol levels rise
- Blood pressure rises
- Glucose tolerance is reduced
- Greater amygdala activation
- Lower prefrontal cortical activity
- Increased negative mood

Neurotransmitters

Serotonin helps to initiate sleep. **Acetylcholine** (ACh) is higher during REM sleep (associated with erections in men). **Norepinephrine** (NE) is lower during REM sleep.

- Ratio of ACh and NE is biochemical trigger for REM sleep.
- NE pathway begins in the pons, which regulates REM sleep.

Dopamine produces arousal and wakefulness. Dopamine levels rise upon waking.

Orexin (or hypocretin) is a neurotransmitter that regulates arousal, wakefulness, and appetite. Activation of orexin triggers wakefulness, while low levels of orexin at night serve to drive sleep. A deficiency of orexin results in sleep-state instability, leading to sleep disorders like narcolepsy.

Melatonin is converted from serotonin in the pineal gland in the brain under directions from the body's internal circadian clock.

- Melatonin production is inhibited by activation of photoreceptors cells in the retina.
- Melatonin production increases in the evening, causing drowsiness.
- Melatonin secretion regulates the sleep-wake cycle by inhibiting the circadian alerting system in the suprachiasmatic nucleus.
- Bright environmental light, capable of suppressing human melatonin, reverses the winter depressive symptoms of patients with seasonal affective disorder (SAD).

Drugs That Alter Sleep

Dopamine increases wakefulness. Dopamine blockers (e.g., antipsychotics) increase sleep.

Benzodiazepines cause limited decrease in REM and Stage 4 sleep. If used chronically and then stopped, sleep latency will increase.

- Moderate alcohol consumption leads to early sleep onset and increased wakefulness during the second half of the night. Intoxication decreases REM; REM rebound (with nightmares) occurs during withdrawal.
- Barbiturates decrease REM; REM rebound, including nightmares, occurs in withdrawal.
- Major depression increases REM, decreases REM latency (45 rather than 90 minutes), and decreases Stages 3 and 4 sleep. It also leads to early morning waking and multiple awakenings during the night.

SLEEP DISORDERS

Narcolepsy

Narcolepsy is a neurological disorder that decreases the ability to control the sleep-wake cycle. It is a REM disorder; patients typically enter REM within 10 minutes. It is linked to a deficiency in hypocretin.

Narcolepsy patients experience 4 main symptoms (narcoleptic tetrad):

- Sleep attacks and excessive daytime sleepiness (**most common** symptoms)
- Cataplexy (pathognomonic sign): sudden loss of consciousness and symptoms ranging from slurred speech to total body collapse (can be triggered by loud noise, emotions, etc.)
- Hypnopompic and hypnogogic hallucinations (common)
- Sleep paralysis: the inability to move or speak while falling asleep or waking up from sleep

Treatment is modafinil or psychostimulants to treat the sleepiness and an antidepressant to treat the cataplexy.

Sleep Apnea

With sleep apnea, individuals have episodes of apnea during the night causing them to have difficulties breathing. It is characterized by a loud snore and common in obese, middle-aged men.

- **Obstructive or upper airway sleep apnea**: airway collapses or becomes blocked during sleep
- **Central sleep apnea**: area of the brain that controls breathing does not send the correct signals to the breathing muscles; often associated with medical problems
- **Mixed sleep apnea**: patients experience both obstructive and central sleep apnea

Clinical presentation includes:

- High risk of sudden death during sleep
- Development of severe nocturnal hypoxemia
- Pulmonary and systemic hypertension (with elevated diastolic pressure)
- Nocturnal cardiac arrhythmias (potentially life-threatening)
- Bradycardia, then tachycardia

Symptoms commonly include dry mouth, headaches, and daytime tiredness. Restlessness and loud snoring are typically reported by sleep partners.

To diagnose, patients are referred to nocturnal polysomnography or home sleep tests to monitor pulse, oxygen level, etc. Treatment may be limited to diet or smoking cessation (mild cases); CPAP (continued positive airway pressure) in moderate to severe cases; and surgery in cases due to obstructive reasons.

Insomnia

Insomnia is characterized by difficulty initiating and maintaining sleep (DIMS).

- **Primary insomnia**
- **Secondary insomnia** (most common) is caused by medical problems, psychiatric problems, medications, etc.

Symptoms of insomnia include sleepiness during the day, general tiredness, irritability, and problems with concentration or memory.

Treatment varies:

- Sleep hygiene
- Behavior modification: stimulus control
- Pharmacotherapy: zaleplon, zolpidem, eszopiclone (work on sleep receptors to help individual to fall and stay asleep)
- Ramelteon, a melatonin receptor-agonist, works on the sleep wake cycle and has less incidence of dependence

Table 8-2. Parasomnias

Diagnosis	Sleep Stage	Features	Treatment	Memory for Event
Night terror	3	• Common in young boys • Familial • Wake up in middle of night and scream	Benzodiazepines	No
Nightmares	REM	Common during stressful times	Antidepressants	Yes
Somnambulism (sleep-walking)	3	• Confused and disoriented if awakened • Common in children • May harm themselves	Benzodiazepines	N/A
Bruxism (teeth grinding)	2	Usually stress-related	Teeth guards	N/A

Psychiatric (DSM-5) Disorders

9

Learning Objectives

- ❑ Demonstrate understanding of the different psychiatric disorders that are identified in childhood and adolescence
- ❑ Demonstrate understanding of the different thought disorders that affect interpretation of reality
- ❑ Demonstrate understanding of the different mood and anxiety disorders
- ❑ Answer questions about the features of obsessive-compulsive disorder
- ❑ Answer questions about different eating disorders
- ❑ Answer questions about somatic symptom, dissociative, and personality disorders
- ❑ Demonstrate understanding about the types of sexual disorders

CHILDHOOD AND ADOLESCENCE

Intellectual Disability

The most common known cause of intellectual disability is **fetal alcohol syndrome** (FAS), while the most common genetic causes are **Down** and **fragile-X syndromes**.

Table 9-1. Intellectual Disability Levels

Level	Functioning
Mild (85% of intellectually disabled; 2:1 male:female)	Self-supporting with some guidance; usually diagnosed first year in school
Moderate	Benefits from vocational training but needs supervision; sheltered workshops
Severe	Vocational training not helpful, can learn to communicate and manage basic self-care habits
Profound	Needs highly structured environment with constant nursing care and supervision

Autism Spectrum Disorders

The hallmark of autism spectrum disorders is an **inability to connect with others**. It is usually diagnosed age <3. Boys > girls.

Clinical features include:

- Problems with communication and reciprocal social interaction
  - Abnormal or delayed language development; impairment in verbal and non-verbal communication
  - No separation anxiety; problems with relationships
  - Pronoun reversal
  - Fails to assume anticipatory posture, shrinks from touch
  - Poor eye contact
- Restrictive and repetitive behaviors (RRBs), interests, or activities
  - Stereotype or repetitive movements (echolalia, lining up toys)
  - Inflexibility
  - Oblivious to external world
  - Preference for inanimate objects

With autism, monozygotic concordance is greater than dizygotic concordance. Severity correlates to IQ deficiency. EEG may be abnormal. Seizures are present in 25% of patients.

Differential diagnosis includes:

- Rett syndrome
  - Girls > boys
  - Microcephaly
- Social communication disorder
  - Communication disorder
  - Absence of RRBs

Treatment is behavioral techniques (shaping) and antipsychotics (for aggression only), e.g., risperidone.

Tourette Syndrome

Tourette syndrome is characterized by multiple motor and vocal tics that occur many times per day or intermittently for >1 year. Men > women 3:1.

- Mean onset is age 7 (onset must be age <18)
- Tics can be simple (rapid, repetitive contractions) or complex (appear as more ritualistic and purposeful); simple tics appear first
- Evidence of genetic transmission: ~50% concordance in monozygotic twins
- Associated with increased levels of dopamine
- Associated with ADHD and OCD

Treatment is haloperidol, pimozide, or clonidine.

Attention Deficit Hyperactivity Disorder

Attention deficit hyperactivity disorder (ADHD) is marked by inattention, impulsivity, and/or hyperactivity that lead to problems functioning at home, school, or work. Men > women 10:1.

Impairment must occur in at least 2 settings.

- Symptoms >6 months
- Symptoms age <12
- Associated with a dopaminergic and noradrenergic imbalance

Treatment is methylphenidate, dextroamphetamine, atomoxetine, or guanfacine.

Table 9-2. Conduct vs. Oppositional Defiant Disorder

	Conduct Disorder	Oppositional Defiant Disorder
Age	Until age 18	Preteens–teens
Gender	Boys > girls	Boys > girls (pre-puberty)
		Boys = girls (post-puberty)
Symptoms	• 6 months of aggressive behavior • Violation of rules of society • Destruction of property • Deceitfulness or theft	• 6 months of negative, hostile, and defiant behaviors toward authority figures
Etiology	Genetic	

THOUGHT DISORDERS

Thought disorders are disorders that affect one's thinking, behavior, and interpretation of reality.

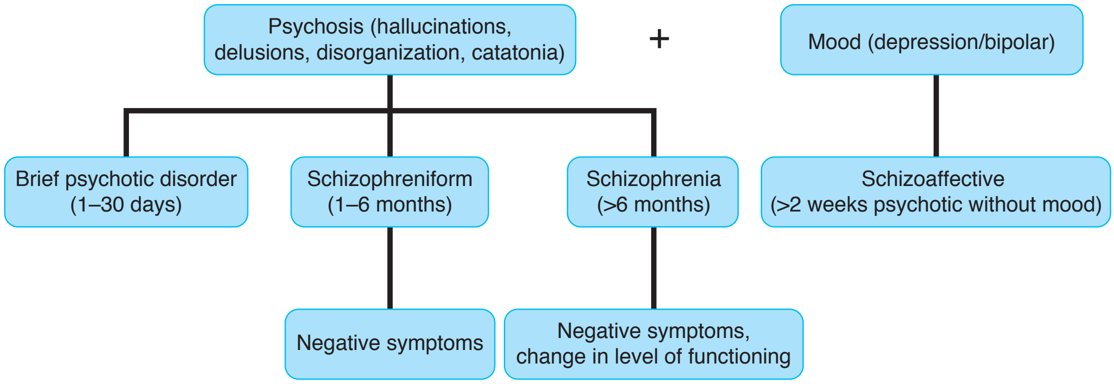


Figure 9-1. Thought Disorders

Table 9-3. Classification of Thought Disorders

Diagnosis	Symptoms	Duration of Symptoms	Treatment
Brief psychotic disorder	Hallucinations, delusions, disorganized behavior or thinking	>1 day but <30 days	Antipsychotics
Schizophreniform disorder	Hallucinations, delusions, disorganized behavior or speech	>1 month but <6 months	Antipsychotics
Schizophrenia	Hallucinations, delusions, disorganized behavior or speech, catatonic symptoms, negative symptoms, marked reduction in level of functioning	>6 months	Antipsychotics
Schizoaffective disorder	• Schizophrenia symptoms such as hallucinations and delusions PLUS mood disorder symptoms such as depression and mania • Must have at least 2 weeks of psychotic symptoms in the absence of mood symptoms		Antipsychotics and mood stabilizers if bipolar type or antidepressants if depressed type

Schizophrenia

Schizophrenia is seen in 1% of population. Men = women.

- Age of onset: men age 15–25; women age 25–35 (90% of patients with schizophrenia are age 15–55).
- Persons with schizophrenia have higher mortality rate from accident and natural causes.
- Persons with schizophrenia are more likely to have been born in winter or early spring (increased risk of schizophrenia after exposure to influenza).
- Lifetime prevalence of substance abuse >50%.
- >90% of schizophrenics smoke (nicotine may reduce positive symptoms and improve some cognitive impairments).

There are several theories of schizophrenia:

- **Genetic**: monozygotic greater than dizygotic
- **Viral**: more born in winter and early spring
- **Social**: downward drift and social causation theories
- **Neurochemical/biochemical**: associated with increased levels of dopamine

Table 9-4. Biochemical Theories of Schizophrenia

Neurotransmitter	Dopamine	Serotonin	Glutamate	Nicotine	GABA
Change	Increase	Increase	Increase/decrease	Decrease	Decrease
Effects	Positive symptoms	Positive and negative symptoms	PCP (glutamate antagonist) leads to schizophrenia-like symptoms	Cognition	Leads to hyperactivity of dopaminergic neurons

Table 9-5. Schizophrenia-Associated Neuropathology

Brain Area	Ventricles	Symmetry	Limbic System	Prefrontal Cortex	Thalamus	Basal Ganglia and Cerebellum
Change	Increase	Decrease	Decrease	Decrease	Decrease	Increase/decrease

MOOD DISORDERS

Major Depressive Disorder

- Women > men
- Symptoms >2 weeks and affect level of functioning (must include depressed mood or anhedonia, plus other symptoms such as low energy, poor concentration, sleeplessness, loss of appetite or libido, suicidal ideation)
- Associated with decreased levels of NE, dopamine, and serotonin
- Suicide rate 15%

Treatment is antidepressants.

Major depressive disorder with seasonal pattern is associated with abnormalities in melatonin. It is common in the Northern hemisphere during the winter months. Treatment is bright light therapy (phototherapy).

Persistent Depressive Disorder

Persistent depressive disorder is less severe than major depressive disorder.

- Symptoms >2 years; depressive symptoms experienced most days
- Hospitalization not usually needed; functioning not significantly impaired

Treatment is primarily psychotherapy.

Bipolar Disorder

Bipolar disorder is the **most genetic of all the psychiatric disorders**. Men = women.

- Symptoms of mania >1 week, with loss of functioning.
  - Manic symptoms include increased energy, decreased sleep, euphoria, delusions of grandeur, increased libido, distractibility, flight of ideas, increased self-esteem.
- Symptoms of major depressive disorder are very common but not necessary for diagnosis.

There are 2 types of bipolar disorder: **bipolar I** involves **mania and depression**; **bipolar II** involves **hypomania and depression**.

Table 9-6. Bipolar Disorder Classification

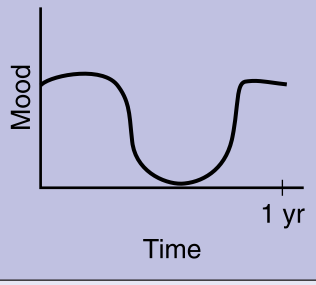
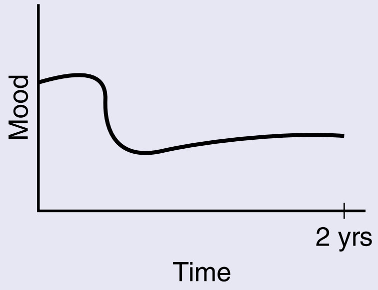
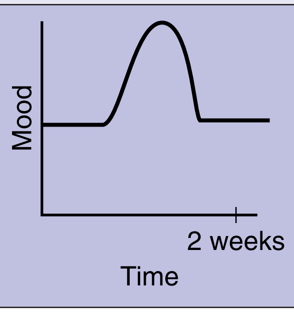
	Mania	Hypomania
Symptoms	Severe	Less severe
Level of functioning	Not functional	Functional
Hospitalization	Yes	No

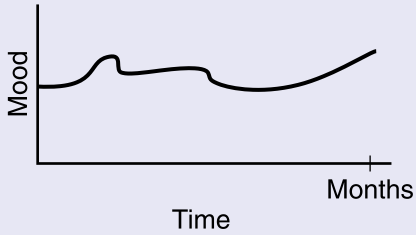
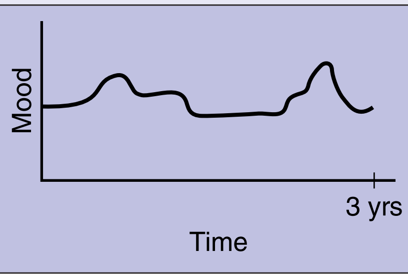
Cyclothymic Disorder

- Symptoms >2 years characterized by:
  - Mood swings
  - Periods of hypomania alternating with periods of milder depression (neither meets criteria for mania or major depressive disorder)

Treatment is primarily psychotherapy.

Table 9-7. Mood Disorders

Diagnosis	Duration	Mood Chart
Major depressive disorder	2 weeks	
Persistent depressive disorder	2 years	
Bipolar I disorder (mania)	1 week	

Diagnosis	Duration	Mood Chart
Bipolar II disorder	Months	
Cyclothymic disorder	2 years	

ANXIETY DISORDERS

Anxiety disorders are linked to abnormalities in serotonin, norepinephrine, and GABA. Women > men (especially young women).

Panic Disorder

- Presence of panic attacks for >1 month
- Involves worry about having more attacks and significant maladaptive behavioral changes related to the attack
- Panic attacks are short-lived and out of the blue, with increased autonomic hyperactivity:
  - Increased pulse
  - Hyperventilation
  - Palpitations
  - Tremors
  - Diaphoresis
  - Dissociative symptoms

Treatment is benzodiazepines (for panic attack) or SSRIs (for panic disorder). If hyperventilating, the patient should be instructed to breathe into a paper bag.

Generalized Anxiety Disorder

Generalized anxiety disorder is a constant sense of worry for >6 months about things one should not have to worry about. The anxiety interferes with daily life:

- Problems with sleep
- Decreased concentration
- Irritability

Treatment is SSRIs and buspirone.

Phobias

Phobias are irrational fears of things or situations and the need to avoid them. Specific phobias include fear of things or objects, such as animals, heights, and elevators.

Treatment is a behavioral modification technique such as systematic desensitization or flooding.

Social Anxiety

Social anxiety is fear of being embarrassed or humiliated in a social situation, such as in a public restroom or restaurant, or public speaking (called stage fright or **performance anxiety** only if related to performance in public).

Treatment is antidepressants and beta-blockers (for stage fright, given before an event).

OBSESSIVE-COMPULSIVE DISORDER AND RELATED DISORDERS

Obsessive-Compulsive Disorder

- **Obsessions:** thoughts that are intrusive, senseless and time consuming
  - Ego-dystonic
  - Thoughts are distressing
  - Increases anxiety
  - Most common thoughts include contamination and doubt
  - Defense mechanism: reaction formation
- **Compulsions:** acts that are repetitive, time consuming
  - Ego-dystonic
  - Reduces the anxiety associated with the obsessive thoughts
  - Most common include washing and checking
  - Defense mechanism: undoing
- Equal incidence in men and women

Treatment is antidepressants and behavioral modification (exposure and response prevention).

Body Dysmorphic Disorder

Body dysmorphic disorder is belief that some part of one's body is abnormal, defective, or misshapen. It is associated with serotonin. It must be differentiated from body image disturbance seen in anorexia nervosa.

Treatment is psychotherapy and SSRIs.

Hoarding Disorder

Hoarding disorder is difficulty parting with one's possessions regardless of their value; level of functioning changes as a result of clutter in the home. There is distress when thinking of getting rid of items.

Treatment is SSRIs and psychotherapy.

Trichotillomania

Trichotillomania is an irresistible urge to pull out one's own hair followed by a sense of relief. Many patients eat or chew the hair. The most common areas of hair pulling are the scalp, eyebrows, eyelashes, beard, and pubic area.

TRAUMA AND STRESSOR-RELATED DISORDERS

Post-Traumatic Stress Disorder and Acute Stress Disorder

Stress disorders result from exposure to actual or threatened death, serious injury, or sexual violation in one of the following ways:

- Direct experience
- Repeated exposure (first responders)
- Witnessing the event
- Learning of violent or accidental traumatic event involving a close family member or friend

Exposure may lead to re-experiencing of symptoms in the form of nightmares or flashbacks. Women > men. Symptoms include:

- Phobic avoidance
- Hypervigilance
- Increased startle reflex
- Mood instability
- Sleep disturbances
- Dissociative symptoms

Treatment is exposure therapy and SSRIs.

	Post-Traumatic Stress Disorder	Acute Stress Disorder
Onset	Anytime	>3 days, <1 month
Duration	≥1 month	<1 month

Adjustment Disorder

Adjustment disorder is a maladaptive response to an identifiable stressor causing distress in functioning.

- Symptoms must occur within 3 months of stressor
- Symptoms cannot last >6 months in duration

Treatment is supportive psychotherapy.

EATING DISORDERS

Anorexia Nervosa

Anorexia is characterized by restriction of food intake that leads to **>15–20% loss of ideal body weight** or **BMI <17**. It is difficult to treat. Girls > boys.

- Body image disturbance (patients feel fat even though they are very thin)
- Fear of gaining weight
- Poor sexual adjustment
- Medical complications include:
  - Abnormal electrolytes
  - Lanugo hair
  - Abnormal hormones
  - Low blood pressure
  - Heart failure
  - Osteoporosis

Treatment is hospitalization, behavioral modification, SSRIs, and family therapy.

Bulimia Nervosa

Bulimia nervosa is characterized by binge eating followed by purging. **Weight is normal**. Most patients recover. Girls > boys.

In bulimia, defense mechanisms are involved, e.g., purge: undoing. Characterized by:

- **Binge** (rapid ingestion of food)
- **Purge** (compensatory behavior)
  - **Vomiting** (signs of **repetitive emesis** include abrasions and callous in fingers/hands, esophageal tears, enlarged parotid glands, and dental cavities)
  - Exercising
  - Fasting
  - Use of laxatives
  - Use of diuretics

Treatment is behavioral modification and SSRIs.

Binge Eating Disorder

Binge eating disorder is binge eating without the compensatory behavior seen in bulimia nervosa. **Weight is above normal**.

Treatment is stimulants.

SOMATIC SYMPTOM AND RELATED DISORDERS

Somatic Symptom Disorder

- Excessive thoughts, feelings, and behavior related to the somatic symptoms
- Duration >6 months
- Functioning impaired
- May be only one symptom; could be related to medical illness
- Main focus is how you react to the symptom

Treatment should be by one identified physician along with psychotherapy.

Illness Anxiety Disorder

Illness anxiety disorder is characterized by the belief that one has an underlying illness, despite constant reassurance.

- Duration >6 months
- Somatic symptoms are not present; if present, mild

Treatment is psychotherapy.

Conversion Disorder

Conversion disorder is the development of neurological symptoms following a psychological stressor that cannot be medically explained.

- All work-up tests will be negative.
- Patients will be indifferent to symptoms *(la belle indifference)*.

Treatment is psychotherapy.

Factitious Disorder

Factitious disorder is the conscious production of signs and symptoms of a mental or physical illness. There are 2 types: **imposed on self** and **imposed on others**.

- Unconscious motivation (without knowing why)
- No obvious external gains
- Most patients become angered when confronted, will leave hospital against medical advice

Treatment is psychotherapy.

Malingering

Malingering is **not a mental illness**. It is the conscious production of signs and symptoms of a mental or physical illness.

- Conscious motivation
- Obvious external gains: money, avoiding prison, time off from work or school
- Most patients become angered when confronted

Table 9-8. Somatic Symptom Disorder vs. Factitious Disorders and Malingering

	Somatic Symptom	Factitious	Malingering
Symptom production	Unconscious	Intentional	Intentional
Motivation	Unconscious	Unconscious	Intentional

DISSOCIATIVE DISORDERS

In dissociative disorders, the defense mechanism used is **dissociation**. It involves the splitting off of brain from consciousness, typically caused by traumatic events.

- **Amnesia**: inability to recall important personal information
  - Dissociative identity disorder (multiple personality): presence of ≥2 distinct identities; will have lapses in memory
- **Depersonalization disorder**: recurrent experiences of being detached from or outside of one's body
  - "Out of body" experiences
  - Reality testing stays intact
  - Causes significant impairment
- **Fugue** (may appear with all subtypes): involves sudden unexpected travel, an inability to recall one's past, or confusion of identity

PERSONALITY DISORDERS

Personality disorders are maladaptive patterns of behavior. They are ego-syntonic and lifelong.

Cluster A: Odd, Eccentric Type

Paranoid: long-standing suspiciousness or mistrust of others; a baseline of mistrust

- Preoccupied with issues of trust
- Reluctant to confide in others
- Reads hidden meaning into comments or events
- Carries grudges

Schizoid: lifelong pattern of social withdrawal; they like it that way

- Seen by others as eccentric, isolated, and withdrawn
- Restricted emotional expression

Schizotypal: very odd, strange, weird

- Magical thinking (including ESP and telepathy)
- Ideas of reference
- Illusions
- Socially anxious
- Lacks close friends, socially isolated
- Incongruous affect

- Odd speech
- May have short-lived psychotic episodes

Cluster B: Dramatic and Emotional

Histrionic: colorful, dramatic, and extroverted

- Unable to maintain long-lasting relationships
- Attention-seeking
- Desires spotlight
- Uses seductive behavior

Narcissistic: grandiose sense of self-importance

- Preoccupied with fantasies of unlimited wealth, power, love
- Demands constant attention
- Has fragile self-esteem
- Prone to depression
- Meets criticism with indifference or rage
- Genuinely surprised and angered when others don't do as they want
- Can be charismatic

Borderline: very unstable affect, behavior, self-image

- In constant state of crisis, chaos
- Self-detrimental impulsivity: promiscuity, gambling, overeating, substance-related disorders
- Unstable but intense interpersonal relationships
- Have great difficulty being alone
- Self-injurious behavior
- Multiple suicide attempts
- History of sexual abuse
- Defense mechanisms: splitting, passive–aggression
- Women > men 2:1

Antisocial: unable to conform to rules of society (**only personality disorder that requires age 18 for diagnosis**)

- Criminal acts: delinquency, theft
- Truancy
- Running away
- Unable to hold a job
- Unable to maintain enduring attachments
- Reckless
- Aggressive
- Show lack of remorse
- Men > women

Cluster C: Anxious and Fearful

Avoidant: extreme sensitivity to rejection; sees self as socially inept

- Excessive shyness, high anxiety levels
- Social isolation, but an intense, internal desire for affection and acceptance
- Tends to stay in same job, same life situation, and same relationships

Obsessive-compulsive: characterized by orderliness and perfectionism

- Inflexible
- Control freak
- Loves lists, rules, order
- Does not want change
- Rigid and excessively stubborn
- Wants to keep routine

Dependent: gets others to assume responsibility

- Subordinates own needs to others
- Unable to express disagreement
- Greatly fearful of having to care for self
- May be linked to abusive spouse

SEXUAL DISORDERS

Sexual Desire Disorders

In **male hypoactive sexual desire disorder**, men experience a deficiency or absence of fantasies or desires. Reasons: low testosterone, CNS depressants, depression, marital discord; common post-surgery.

Sexual Arousal Disorders

In **female sexual interest/arousal disorder**, women are unable to achieve adequate vaginal lubrication. Reasons include possible hormonal connection (many women report peak sexual desire just prior to menses) and antihistamine/anticholinergic medications, which can reduce vaginal lubrication.

Male erectile disorder (impotence) has 10–20% lifetime prevalence; point prevalence is 3%. Half of men treated for sexual disorder complain of impotence.

- Incidence is 8% young adult and 75% men age >80
- 50% more likely in smokers

Be sure to check alcohol usage, diabetes, and marital conflict, as it must be determined whether the cause is organic or psychological. Assessment is made with the postage stamp test, snap gauge (to test physiological vs. psychological).

Treatment is sildenafil, vardenafil, and tadalafil.

Orgasm Disorders

Female orgasm disorder is an inability to achieve orgasm. Overall prevalence from all causes is 30%.

About 5% of married women age >35 have never achieved orgasm.

Treatment is fantasy, vibrators.

In **premature ejaculation**, the man ejaculates before or immediately after entering vagina. It is more common if early sexual experiences were in backseat of car or with a prostitute, or there is anxiety about the sexual act.

Treatment is stop-and-go technique, squeeze technique, and SSRIs.

Paraphilic Disorders

- Pedophilia: sexual urges toward children; most common paraphilia
- Exhibitionism: recurrent desire to expose genitals to stranger
- Voyeurism: sexual pleasure from watching others who are naked, grooming, or having sex; begins early in childhood
- Sadism: sexual pleasure derived from others' pain
- Masochism: sexual pleasure derived from being abused or dominated
- Fetishism: sexual focus on objects, e.g., shoes, stockings; transvestite fetishism involves fantasies or actual dressing by heterosexual men in female clothes for sexual arousal
- Frotteurism: male rubbing of genitals against fully clothed woman to achieve orgasm; subways and buses
- Zoophilia: animals preferred in sexual fantasies or practices
- Coprophilia: combining sex and defecation
- Urophilia: combining sex and urination
- Necrophilia: preferred sex with cadavers
- Hypoxyphilia: altered state of consciousness secondary to hypoxia while experiencing orgasm; achieved with autoerotic asphyxiation, poppers, amyl nitrate, nitric oxide

Genito-Pelvic Pain Disorders

Genito-pelvic pain/penetration disorders involve involuntary muscle constriction of the outer third of the vagina, which prevents penile insertion. Psychological in origin, they involve recurrent and persistent pain before, during, or after intercourse.

- Diagnosed only in women
- Not diagnosed if caused by a medical conditions

Treatment is relaxation and Hegar dilator.

Psychopharmacology 10

Learning Objectives

- ❑ Define the side effect profile of different receptors
- ❑ Demonstrate understanding of the types of antipsychotics and how they work
- ❑ Demonstrate understanding of the types of antidepressants and how they work
- ❑ Demonstrate understanding of the types of mood stabilizers and how they work
- ❑ Demonstrate understanding of the types of anxiety medications and how they work

SIDE EFFECT PROFILE

Receptor	Effects
Histamine	Sedation, weight gain
Muscarine	Anticholinergic (dry mouth, blurry vision, constipation, confusion, etc.)
Alpha 1	Dizziness, hypotension

ANTIPSYCHOTIC (NEUROLEPTIC) MEDICATIONS

Antipsychotic medications are used to treat 2 types of conditions:

- Schizophrenia and other psychotic disorders
- Hiccups, Tourette syndrome, and bipolar disorders

The **mechanism of action** is dopamine blockage at the postsynaptic receptors.

Table 10-1. Medication Side Effects

Side Effect	Peak	Treatment
Dystonic reaction	Hours to days	Anticholinergics: benztropine, trihexyphenidyl, diphenhydramine
Rigidity	3 weeks	Lower dose or anticholinergics
Tremors	6 weeks	Lower dose or anticholinergics
Akathisia	10 weeks	B-blockers, benzodiazepines; lower dose or switch to atypical
Tardive dyskinesia	>3–6 months	Switch to atypical or clozapine
Neuroleptic malignant syndrome	Any time	May be lethal; dantrolene or bromocriptine

Dopamine tracts include:

- **Mesolimbic/mesocortical**: reduces psychotic symptoms
- **Nigrostriatal**: increases movement disorder
- **Tuberoinfundibular**: increases prolactin (galactorrhea, amenorrhea, gynecomastia)

Types of Antipsychotics

Table 10-2. Typical vs. Atypical Antipsychotics

Typical	Atypical
Dopamine	Dopamine and serotonin
Treats mostly positive symptoms	Treats positive and negative symptoms
More side effects	Fewer side effects

The potency of typical antipsychotic medications is as follows:

Potency	Extrapyramidal Symptoms	Anticholinergic Effects
High (haloperidol)	High	Low
Low (chlorpromazine)	Low	High

Typical antipsychotics have movement and prolactin side effects.

- Haloperidol
- Fluphenazine
- Chlorpromazine

Note

Extrapyramidal reactions include:

- Choreiform: jerky movements
- Athetoid: slow, continuous movements
- Rhythmic: stereotypical movements

- Mesoridazine
- Thioridazine (additional increased risk of retinitis pigmentosa and retrograde ejaculation)

Atypical antipsychotics are known for weight gain, increased risk of diabetes, and metabolic syndrome, but also cause movement and prolactin side effects.

- Clozapine (additional increased risk of agranulocytosis (<1%), seizures, drooling)
- Risperidone
- Olanzapine
- Quetiapine
- Ziprasidone
- Aripiprazole (partial dopamine agonist)
- Paliperidone
- Lurasidone

ANTIDEPRESSANT MEDICATIONS

Antidepressant medications are used to treat depression, anxiety, and pain disorders. The **mechanism of action** is on the norepinephrine (NE), serotonin (5-HT), and dopamine receptors.

Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) block reuptake of serotonin and norepinephrine, alpha-1 adrenergic receptors, and muscarinic receptors. They cause many side effects and are lethal in overdose.

- Amitriptyline
- Imipramine (monitor levels)
- Nortriptyline (monitor levels)
- Desipramine (monitor levels)
- Clomipramine

Monoamine Oxidase Inhibitors

Monoamine oxidase inhibitors (MAOIs) are the treatment of choice for major depressive disorder with atypical features. The **mechanism of action** is inhibition of MAO, an enzyme that metabolizes serotonin, epinephrine, and NE.

- Phenelzine
- Tranylcypromine
- Isocarboxazid

Note

Use TCAs with caution in the elderly, as they cause many side effects.

MAOI plus tyramine can cause **hypertensive crisis**. Signs include occipital headache, stiff neck, nausea/vomiting, chest pain, dilated pupils, nosebleed, and elevated blood pressure.

- Problem foods: aged cheese, dried fish, sauerkraut, sausage, chocolate, avocados, red wine
- Safe foods: cottage cheese, some wine (mostly white)

Selective Serotonin Reuptake Inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are the **most commonly used antidepressants**. The **mechanism of action** is inhibition of the reuptake of serotonin. They are also used to treat anxiety and sexual disorders.

- Fluoxetine
- Sertraline
- Paroxetine
- Citalopram
- Escitalopram
- Fluvoxamine

Side effects include weight gain, sexual problems, headaches, and GI complaints.

Note

SSRIs do not affect histamine, alpha, or muscarinic receptors, so their side effect profile is cleaner than the other antidepressants.

Serotonin syndrome is associated with high doses, MAOI/SSRI combo, and MAOI/synthetic narcotic combination. Symptoms include general restlessness, sweating, insomnia, nausea, diarrhea, cramps, delirium, and myoclonus.

Treatment is removal of the causative agent, stopping the SSRI, and administering cyproheptadine.

Serotonin Norepinephrine Reuptake Inhibitors

Serotonin norepinephrine reuptake inhibitors (SNRIs) are used to treat depression, anxiety, and pain disorders.

The **mechanism of action** is inhibition of the reuptake of serotonin and norepinephrine.

- Venlafaxine
- Desvenlafaxine
- Duloxetine

Side effects include increased blood pressure and blurry vision (common).

Other Antidepressants

- Trazodone
  - 5-HT receptor antagonist, alpha-1 blocker
  - Almost no anticholinergic adverse effects
  - May lead to priapism so sometimes used to treat erectile dysfunction
  - Sedation is common side effect

- Mirtazapine
  - Stimulates NE and 5-HT release; blocks 5-HT2 and 5-HT3 receptors
  - Sedation and weight gain are common side effects
- Bupropion (approved for depression and smoking cessation)
  - Relatively weak inhibitor of the neuronal reuptake of norepinephrine and dopamine; does not inhibit the reuptake of serotonin
  - Not associated with weight gain or sexual side effects
  - Side effect is increased risk of seizures, so it is not used in patients with seizure disorders, eating disorders, or alcohol withdrawal seizures

MOOD STABILIZER MEDICATIONS

Lithium

Lithium is the drug of choice for bipolar disorders. It is quickly absorbed from the GI tract—not protein-bound or metabolized. Therapeutic index is low, meaning it requires reaching plasma levels very close to toxic levels for full effect (reached in 10–14 days).

Good kidney function and adequate salt- and fluid-intake are essential with lithium, as 95% is excreted in urine.

The **mechanism of action** (hypothesized) is blocking of inositol-1-phosphate (second messenger).

- Monitor blood levels; if >2.5 meq/L, consider dialysis as the treatment of choice.
- Potassium-sparing diuretics have no effect; loop diuretics will produce increased serum levels.
- Side effects include:
  - Tremor, thirst, anorexia, GI distress
  - Polyuria, polydipsia, edema
  - Acne
  - Hypothyroidism
  - Nephrotoxicity
  - Teratogenicity (Ebstein's anomaly affecting the tricuspid valve)
  - Diabetes insipidus
  - If toxic, ataxia, seizures and confusion may be seen.

Valproic Acid

Valproic acid is used to treat bipolar disorders and rapid cycling bipolar disorders. The **mechanism of action** involves augmentation of GABA in CNS.

- Side effects include sedation, weight gain, tremors, alopecia, GI distress, and teratogenicity (neural tube defects).

- If toxic, may cause confusion, coma, or cardiac arrest.
- Monitor blood levels, as it can cause hepatotoxicity (liver function impairment).

Carbamazepine

The **mechanism of action** involves blocking sodium channels in neurons with action potential; it alters central GABA receptors.

- Side effects include GI distress, rash, mild leukopenia, agranulocytosis, and aplastic anemia.
- If toxic, may cause hypotension, tachycardia, respiratory depression, or coma.
- Monitor blood levels and signs of rash.

Lamotrigine

Lamotrigine is associated with Stevens-Johnson syndrome.

ANTIANXIETY MEDICATIONS

Benzodiazepines

Benzodiazepines are used to treat anxiety disorders, sleep disorders, alcohol withdrawal, and seizures.

The **mechanism of action** is depression of the CNS at the limbic system, RAS, and cortex. Benzodiazepines bind to GABA-chloride receptors, facilitating the action of GABA.

- All benzodiazepines undergo hepatic microsomal oxidation (except for lorazepam, oxazepam, and temazepam, which undergo glucuronide conjugation).
- Side effects include sedation, insomnia, addiction, falls (elderly), confusion, and disinhibition.

Buspirone

Buspirone is used for generalized anxiety disorder and other anxiety disorders when possible abuse of benzodiazepines is a concern. It has no withdrawal effect and is not potentiated by alcohol.

The **mechanism of action** works on serotonin, not on GABA.

- Full effect is seen >7 days
- Some sedation is seen
- Has low-abuse potential

Brain Function and Neurocognitive Disorders

11

Learning Objectives

- ❑ Demonstrate understanding of left and right brain dominance
- ❑ Be able to correlate specific function with corresponding part of the brain
- ❑ Demonstrate understanding of how healthy parts of the brain differ from injured parts
- ❑ Answer questions about how dominant parietal lobe dysfunction differs from non-dominant dysfunction
- ❑ Demonstrate understanding of the different neurotransmitters and how they affect the brain
- ❑ Demonstrate understanding about how the neurocognitive disorders differ

LEFT AND RIGHT BRAIN DOMINANCE

The **left hemisphere** is dominant in **language** and **calculation-type problem solving**. It is dominant in 97% of the population (60–70% in left-handed persons).

- Stroke damage to the left hemisphere is more likely to lead to **depression**.

The **right hemisphere** is dominant in **perception**, **artistic**, and **visual–spatial tasks**. It is activated for intuition-type problem solving.

- Stroke damage to the right hemisphere is more likely to lead to **apathy and indifference**.

AREAS OF THE BRAIN

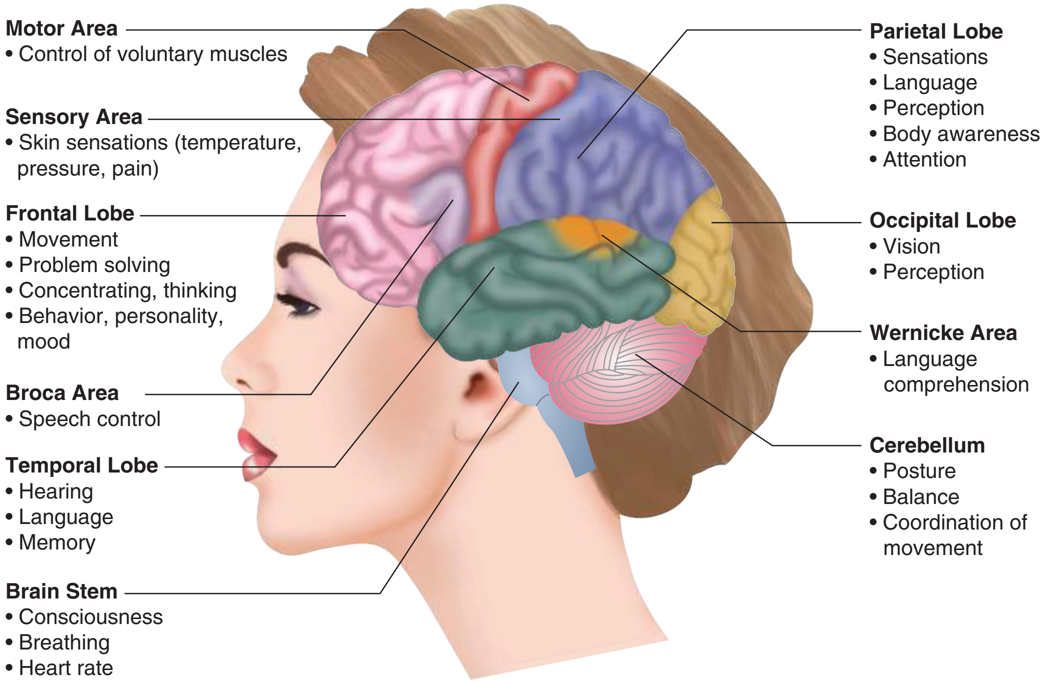


Figure 11-1. Functional Areas of the Brain

	Healthy Brain	Injured Brain
Frontal lobe	• Personality/emotions • Intelligence • Attention/concentration • Judgment • Body movement • Problem-solving • Speech (speaking and writing)	• Loss of movement (paralysis) • Repetition of a single thought • Unable to focus on a task • Mood swings, irritability, impulsiveness • Changes in social behavior and personality • Difficulty problem solving • Difficulty with language; cannot get the words out (aphasia)
Parietal lobe	• Sense of touch, pain, and temperature • Distinguishing size, shape, and color • Spatial perception • Visual perception	• Difficulty distinguishing left from right • Lack of awareness or neglect of certain body parts • Difficulty with eye-hand coordination • Problems reading, writing, naming • Difficulty with mathematics
Occipital lobe	Vision	• Defects in vision or blind spots • Blurred vision • Visual illusions/hallucinations • Problems reading and writing

	Healthy Brain	Injured Brain
Temporal lobe	• Speech (understanding language) • Memory • Hearing • Sequencing • Organization	• Difficulty understanding language and speaking (aphasia) • Difficulty recognizing faces • Difficulty identifying/naming objects • Problems with short- and long-term memory • Changes in sexual behavior • Increased aggressive behavior
Cerebellum	• Balance • Coordination	• Difficulty coordinating fine movements • Difficulty walking • Tremors • Dizziness (vertigo) • Slurred speech
Brainstem	• Breathing • Heart rate • Alertness/consciousness	• Changes in breathing • Difficulty swallowing food and water • Problems with balance and movement

APHASIA

Aphasia is an impairment of language affecting one's ability to speak/understand speech, read, or write.

- **Dominant** (**left**) parietal lobe dysfunction (in most right-handed and some left-handed patients):
  - Language disorders (aphasia, alexia)
  - Gerstmann syndrome (dyscalculia, dysgraphia, finger agnosia, right-left confusion)
  - Apraxia
- **Non-dominant** (**right**) parietal lobe dysfunction:
  - Hemispatial neglect
  - Sensory and visual inattention
  - Constructional and dressing apraxia (more severe for right-sided lesions)

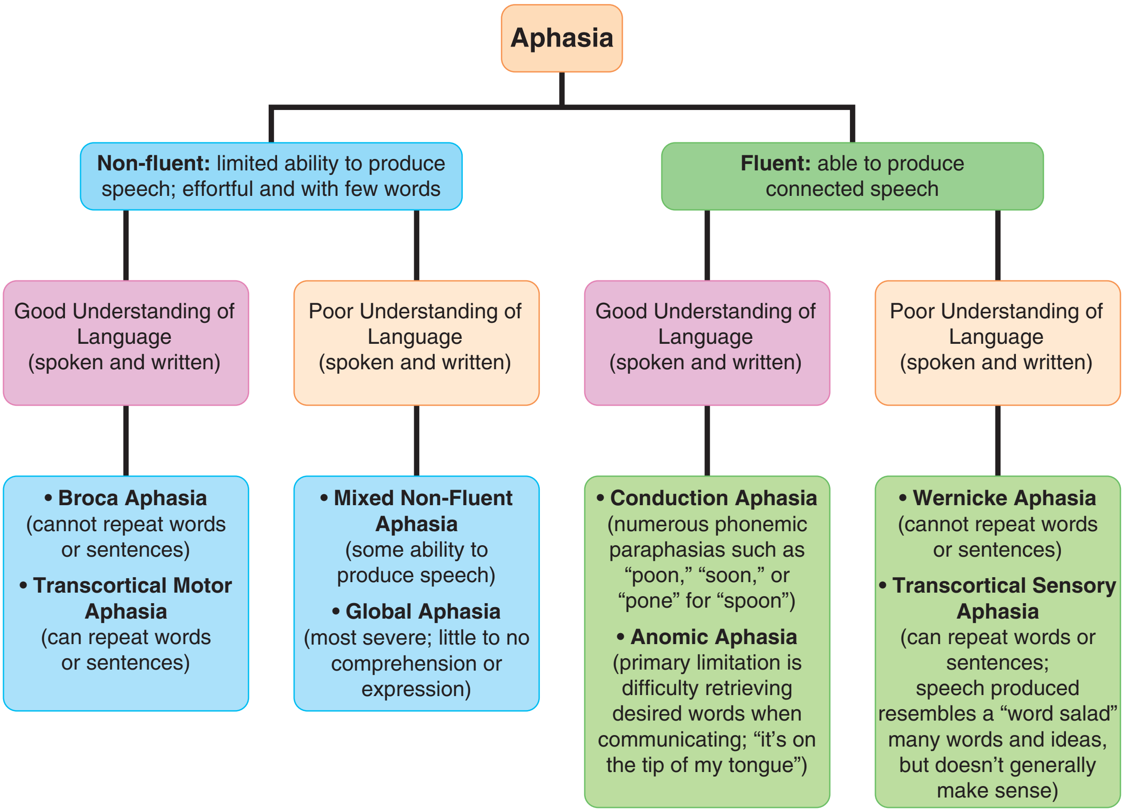


Figure 11-2. Aphasia

NEUROTRANSMITTERS

Acetylcholine (ACh)

ACh is a neurotransmitter at nerve-muscle connections for all voluntary muscles of the body and also many of the involuntary (autonomic) nervous system synapses. The exact role of ACh in the brain is unclear.

- Cholinergic neurons concentrated in the RAS and basal forebrain
- Significant role in Alzheimer disease
- Neurocognitive disorder in general associated with decreased ACh concentrations in amygdala, hippocampus, and temporal neocortex
- Associated with erections in men
- Muscarinic and nicotinic receptors
- In the corpus striatum, ACh circuits are in equilibrium with dopamine neurons

Norepinephrine

Norepinephrine (NE) is one of the catecholamine neurotransmitters. It is a transmitter of the sympathetic nerves of the autonomic nervous system, which mediate emergency response.

- Acceleration of the heart
- Dilatation of the bronchi
- Elevation of blood pressure

NE is implicated in altering attention, perception, and mood. The key pathway is locus ceruleus in upper pons. It is implicated in monoamine hypothesis of affective disorders.

- Depletion of NE leads to depression
- Excess of NE (and serotonin) leads to mania
- Based on 2 observations: reserpine depletes NE and causes depression; antidepressant drugs block NE reuptake, thus increasing the amount of NE available postsynaptically

Receptors:

- Alpha-1: sympathetic (vasoconstriction)
- Alpha-2: on cell bodies of presynaptic neurons, inhibit NE release
- Beta-1: excitatory for heart, lungs, brain
- Beta-2: excitatory for vasodilatation and bronchodilation

Dopamine

Dopamine is the other catecholamine neurotransmitter, synthesized from the amino acid tyrosine.

- D2 receptors most important
- D1 and D5 stimulate G-protein and increase cAMP and excitation
- D2, D3, and D4 inhibit G-protein and decrease cAMP and excitation

Three pathways of known psychiatric importance:

- **Nigrostriatal pathway**: blockade leads to tremors, muscle rigidity, bradykinesia
- **Mesolimbic-cortico pathway**: blockade leads to reduction of psychotic symptoms
- **Tuberoinfundibular system**: blockade leads to increases in prolactin (DA = PIF)

Serotonin (5-Hydroxytryptamine, 5-HT)

Serotonin is the transmitter of a discrete group of neurons that all have cell bodies located in the raphe nuclei of the brain stem. Changes in the activity of serotonin neurons are related to the actions of psychedelic drugs. It is involved in the therapeutic mechanism of action of antidepressant treatments (most are 5-HT reuptake inhibitors; a few new ones are 5-HT agonists).

- Has inhibitory influence; linked to impulse control
- Low 5-HT = low impulse control

- Has role in regulation of mood, sleep, sexual activity, aggression, anxiety, motor activity, cognitive function, appetite, circadian rhythms, neuroendocrine function, and body temperature

Glutamic Acid

Glutamic acid is one of the major amino acids in general metabolism and protein synthesis; it is also a neurotransmitter.

- Stimulates neurons to fire
- Is the principal excitatory neurotransmitter in the brain and the neurotransmitter of neuronal pathways connecting the cerebral cortex and corpus striatum
- Is the transmitter of the granule cells, the most numerous neurons in the cerebellum

There is evidence that glutamic acid is the principal neurotransmitter of the visual pathway. It may have a role in producing schizophrenic symptoms; is the reason for PCP symptoms (antagonist of NMDA glutamate receptors). Glutamate agonists produce seizures in animal studies.

Enkephalins

Enkephalins are composed of 2 peptides, each containing 5 amino acids. They are normally occurring substances that act on opiate receptors, mimicking the effects of opiates. Neurons are localized to areas of the brain that regulate functions influenced by opiate drugs.

Note

There is a new class of antidepressant medications being tested to work on substance P.

Substance P

Substance P is a peptide containing 11 amino acids and is a major transmitter of sensory neurons that convey pain sensation from the periphery, especially the skin, into the spinal cord; also found in numerous brain regions. Opiates relieve pain in part by blocking the release of substance P.

Gamma Aminobutyric Acid

Gamma aminobutyric acid (GABA) is one of the amino acid transmitters in the brain. It occurs almost exclusively in the brain, reduces the firing of neurons, and is the brain's principle inhibitory neurotransmitter (present at 25–40% of all synapses in the brain). GABA is associated with anxiety, cannabis, and benzodiazepines.

NEUROCOGNITIVE DISORDERS

Delirium is an acute onset of impaired cognitive functioning that is fluctuating, brief, and reversible. **Neurocognitive disorder** is a loss of cognitive abilities, impairment of social functioning, loss of memory, and/or change in personality that may be progressive or static. It is reversible only 15% of the time.

Mild neurocognitive disorder is moderate cognitive decline that has minimal interaction with functioning. **Major** neurocognitive disorder is significant cognitive decline that interferes with functioning and independence.

Neurocognitive Disorder Due to Alzheimer Disease

Neurocognitive disorder due to Alzheimer disease is seen in >50% of nursing-home patients and 50–60% of those with neurocognitive disorder.

- Risk factors: female, family history, head trauma, Down syndrome
- Neuroanatomic findings: cortical atrophy, flattened sulci, enlarged ventricles
- Histopathology: senile plaques (amyloid deposits), neurofibrillary tangles, neuronal loss, synaptic loss, granulovacuolar degeneration of neurons
- Associated with chromosome 21 (gene for the amyloid precursor protein)
- Decreased ACh and NE
- Deterioration is gradual; average duration from onset to death ~8 years
- Focal neurologic symptoms rare

Treatment is long-acting cholinesterase inhibitors such as donepezil, rivastigmine, galantamine, and memantine. Antipsychotic medications may be helpful when psychotic symptoms are present but contraindicated to control behavior.

Vascular Neurocognitive Disorder (Multi-Infarct Neurocognitive Disorder)

Vascular neurocognitive disorder is seen in 15–30% of those with neurocognitive disorder.

- Risk factors: male, advanced age, hypertension or other cardiovascular disorders
- Affects small and medium-sized vessels
- Examination may reveal carotid bruits, fundoscopic abnormalities, and enlarged cardiac chambers
- MRI may reveal hyperintensities and focal atrophy suggestive of old infarctions
- Deterioration may be stepwise or gradual, depending on underlying pathology
- Focal neurologic symptoms (pseudobulbar palsy, dysarthria, and dysphagia are most common)
- Abnormal reflexes and gait disturbance often present

Treatment is directed toward the underlying condition and lessening cell damage. Control of risk factors such as hypertension, smoking, diabetes, hypercholesterolemia, and hyperlipidemia is useful.

Table 11-1. Alzheimer Disease vs. Vascular Disorder

Alzheimer	Vascular
Women	Men
Older age	Younger age
Chromosome 21	Hypertension
Linear or progressive deterioration	Stepwise or patchy deterioration
No focal deficits	Focal deficits
Treatment is supportive	Treat underlying condition

Frontotemporal Neurocognitive Disorder (Pick Disease)

- Neuroanatomic findings: atrophy in frontal and temporal lobes
- Histopathology: Pick bodies (intraneuronal argentophilic inclusions) and Pick cells (swollen neurons) in affected areas of brain
- Etiology unknown
- Most common in men with family history of Pick disease
- Difficult to distinguish from Alzheimer disease
- May see features of Klüver-Bucy syndrome (hypersexuality, hyperphagia, passivity)

Neurocognitive Disorder Due to Prion Disease

- Rare spongiform encephalopathy caused by a slow virus (prion)
- Presents with neurocognitive disorder, myoclonus, and EEG abnormalities (e.g., sharp, triphasic, synchronous discharges and, later, periodic discharges)
- Symptoms progress over months from vague malaise and personality changes to neurocognitive disorder and death
- Findings include visual and gait disturbances, choreoathetosis or other abnormal movements, and myoclonus
- Other prions causing neurocognitive disorder (e.g., Kuru) may exist

Neurocognitive Disorder Due to Huntington Disease

- Rare, progressive neurodegenerative disease that involves loss of GABAergic neurons of the basal ganglia; manifested by choreoathetosis, neurocognitive disorder, and psychosis
- Caused by a defect in an autosomal dominant gene located on chromosome 4
- Atrophy of the caudate nucleus, with resultant ventricular enlargement, is common
- Clinical onset ~age 40
- Suicidal behavior fairly common

Neurocognitive Disorder due to Parkinson Disease

- Common, progressive, neurodegenerative disease that involves loss of dopaminergic neurons in the substantia nigra
- Clinical onset ~age 50–65
- Motor symptoms include resting tremor, rigidity, bradykinesia, and gait disturbances
- Neurocognitive disorder occurs in 40% of cases; depressive symptoms common
- Destruction of dopaminergic neurons in the substantia nigra is a key pathogenic component; may be caused by multiple factors including environmental toxins, infection, genetic predisposition, and aging

Treatment of Parkinson disease involves use of dopamine precursors (e.g., levodopa, carbidopa), dopamine agonists (e.g., bromocriptine), anticholinergic medications (e.g., benztropine, trihexyphenidyl), amantadine, and selegiline. Antiparkinsonian medications can produce personality changes, cognitive changes, and psychotic symptoms.

Neurocognitive Disorder with Lewy Bodies

- Hallucinations, parkinsonian features, and extrapyramidal signs
- Antipsychotic medications may worsen behavior
- Patients typically have fluctuating cognition, as well as REM sleep behavior disorder

Neurocognitive Disorder Due to HIV Infection

- HIV directly and progressively destroys brain parenchyma.
- Becomes clinically apparent in at least 30% of those with AIDS, starting with subtle personality changes.
- Diffuse and rapid multifocal destruction of brain structures occurs; delirium is often present.
- Motor findings include gait disturbance, hypertonia and hyperreflexia, pathologic reflexes (e.g., frontal release signs), and oculomotor deficits.
- Mood disturbances in those with HIV infection are apathy, emotional liability, or behavioral disinhibition.

Note

(LBD) 1 yr ← PD → 1 yr (PDD)

Wilson Disease

- Ceruloplasmin deficiency
- Hepatolenticular degeneration
- Kayser-Fleischer rings in the eye
- Asterixis

Normal Pressure Hydrocephalus

- Enlarged ventricles
- Normal pressure
- Neurocognitive disorder, urinary incontinence, and gait apraxia

Treatment is shunt placement.

Pseudodementia

- Typically seen in elderly patients with a depressive disorder who appear to have symptoms of neurocognitive disorder
- Improvement should be seen after treatment with antidepressants
- Onset of symptoms can usually be dated

Table 11-2. Delirium vs. Neurocognitive Disorder

Delirium	Neurocognitive Disorder
Acute onset	Insidious onset
Fluctuating course	Chronic course
Lasts days to weeks	Lasts months to years
Recent memory problems	Recent then remote memory problems
Disrupted sleep-wake cycle	Less disorientation at first
Disorientation	Normal sleep-wake cycle
Hallucinations common	Hallucinations, sundowning
Treat underlying condition	Supportive treatment

Ethics, Law, and Physician Behavior 12

Learning Objectives

- ❑ Demonstrate understanding of how important court cases have shaped medical care
- ❑ Demonstrate understanding of important elements of physician behavior and how they can affect patient care
- ❑ Answer questions about unconscious interactions and how they can affect patient care

LEGAL ISSUES

Selected Important Court Cases

Karen Ann Quinlan: Substituted Judgment Standard

In the Quinlan case, Karen Ann was in a persistent vegetative state, being kept alive only by life support. Her father asked to have her life support terminated according to his understanding of what Karen Ann would want. The court found that "if Karen herself were miraculously lucid for an interval . . . and perceptive of her irreversible condition, she could effectively decide upon discontinuance of the life support apparatus, even if it meant the prospect of natural death."

- The court therefore allowed termination of life support—not because the father asked but because it held that the father's request was most likely the expression of Karen Ann's own wishes.
- Substituted judgment begins with the premise that decisions belong to the competent patient by virtue of the rights of autonomy and privacy.
- In this case, however, the patient is unable to decide and a decision-maker who is the best representative of her wishes must be substituted.
- In legal terms, the patient has the right to decide but is incompetent to do so. Therefore, the decision is made for the patient on the basis of the best estimate of his or her subjective wishes.

The key here is **not who is the closest next of kin,** but **who is most likely to represent the patient's own wishes.**

Brother Fox (*Eichner v. Dillon*): Best Interest Standard

In its decision of *Eichner v. Dillon*, the New York Court of Appeals held that trying to determine what a never-competent patient would have decided is practically impossible. Obviously, it is difficult to ascertain the actual (subjective) wishes of incompetents. Therefore, if the patient has always been incompetent, or no one knows the patient well enough to render substituted judgment, the use of substituted judgment standard is questionable, at best.

Under these circumstances, decisions are made for the patient using the **best interest standard**, the object of which is to decide what a hypothetical "reasonable person" would decide after weighing the benefits and burdens of each course of action.

The issue of who makes the decision is less important here. All persons applying the best interest standard should come to the same conclusions.

Infant Doe: Foregoing Lifesaving Surgery, Parents Withholding Treatment

As a general rule, parents cannot withhold life- or limb-saving treatment from their children. Yet, in this exceptional case they did.

Baby Boy Doe was born with Down syndrome (trisomy 21) and with a tracheoesophageal fistula. The infant's parents were informed that surgery to correct his fistula would have "an even chance of success." Left untreated, the fistula would soon lead to the infant's death from starvation or pneumonia. The parents, who also had 2 healthy children, chose to withhold food and treatment and "let nature take its course."

Court action to remove the infant from his parents' custody (and permit the surgery) was sought by the county prosecutor. The court denied such action, and the Indiana Supreme Court declined to review the lower court's ruling. Infant Doe died at 6 days of age, as Indiana authorities were seeking intervention from the U.S. Supreme Court.

Note that this case is simply an application of the best interest standard. The court agreed with the parents that the burdens of treatment far outweighed any expected benefits.

Roe v. Wade (1973): The Patient Decides

Known to most people as the "abortion legalizing decision," the importance of this case is not limited to its impact on abortion. Faced with a conflict between the rights of the mother and the rights of the putative unborn child, the court held that in the first trimester the mother's rights are paramount and that states may, if they wish, have the mother's rights remain paramount for the full term of the pregnancy.

- Because the mother gets to decide even in the face of threats to the fetus, by extension all patients get to decide about their own bodies and the health care they receive.
- In the United States, the locus for decision-making about health care resides with the patient, not the physician.

Note that courts have held that a pregnant woman has the right to refuse care (e.g., blood transfusions) even if it places her unborn child at risk.

Tarasoff Decision: Duty to Warn and Duty to Protect

A student visiting a counselor at a counseling center in California states that he is going to kill someone. When he leaves, the counselor is concerned enough to call the police but takes no further action. The student subsequently kills the person he threatened. The court found the counselor and the center liable because they did not go far enough to warn and protect the potential victim.

- The counselor should have called the police and then tried in every way possible to notify the potential victim of the potential danger.
- In similar situations, first try to detain the person making the threat. Next, call the police. Finally, notify and warn the potential victim. All three actions should be taken, or at least attempted.

Autonomy

Autonomy is the central principle for ethics in health care. The origins of the word *autonomy* are from the Greek words: "autos" and "nomos," meaning self-rule or self-determination. In ethics, this translates to the principle that every competent individual has the right to make his or her own health care decisions without coercion or coaxing.

In medical practice, competent patients are required to provide informed consent for any treatment or procedure.

Informed Consent

Informed consent is a complete discussion of proper information related to a treatment or procedure between a physician and patient, where the patient voluntarily agrees to the care plan and is free of coercion.

- Full, informed consent requires that the patient has received and understood 5 components of information:
  - Nature of the procedure: What is the procedure or treatment?
  - Purpose or rationale: Why the procedure is being performed? or Why the drug is being administered?
  - Risks of the treatment regimen
  - Benefits of the treatment regimen
  - Alternatives to the recommended treatment regimen
- A signed paper granting informed consent that the patient does not read or does not understand does NOT constitute informed consent:
  - It is not simply enough for a physician to "give" the patient information.
  - The patient needs to understand what he is being treating with, why the physician recommends this treatment, and the risks, benefits, and alternatives to treatment.
  - The patient must understand all 5 components of information.

- The physician cannot discuss treatment options that are not approved. For example, the physician should not discuss with the patient a homeopathic treatment option for cancer.
- Informed consent may be **written or oral**.
- Informed consent can be withdrawn at any time. It does not matter if the patient has signed all the necessary paperwork and is on the way to the operating room; he can decide to not have the procedure done for any reason.

There are 4 situations in which a physician **does not need to obtain informed consent** from a patient in order to perform a procedure or another treatment, i.e., there are special situations in which **informed consent is not required**.

- **Emergency situation**
  - In an emergency situation, the physician should do what is in the best interest of the patient.
  - If the patient is unconscious and needs a life-saving or limb-saving procedure, the procedure should be performed (consent is implied).
- **Waiver is provided by patient**
  - The patient "waives" his right to receive information related to the treatment. In other words, the patient trusts that you will do what is in his best interest.
- **Patient is incompetent**
  - Some patients do not have the capacity to provide informed consent:
    - Are unconscious
    - Have attempted suicide
    - Are in a grossly psychotic or dysfunctional state
    - Are intoxicated with drugs and/or alcohol
    - Are in physical or mental state which prevents simple communication
  - Incompetence is determined by a judge based on a physician's assessment of capacity.
- **Therapeutic privilege**
  - The physician will deprive the patient of autonomy in the interest of health. In other words, if the physician truly believes the patient is not able to make good decisions for himself AND other physicians agree, the physician can treat without informed consent.
  - The physician can invoke therapeutic privilege and move beneficence, nonmaleficence, and justice above patient autonomy.

Committed Patients

A committed mentally ill adult has the following legal rights:

- **Must have treatment available**
  - Patient should be informed on a regular basis what treatment options are available.

- **Can refuse treatment**
  - Patient with a severe form of schizophrenia still has the right to refuse to take antipsychotic medication (except when patients are a danger to themselves or others).
- **Can request a legal hearing to determine *sanity***
  - Competence is a legal matter; only a court can determine competence.
  - Patient has the right to demand a trial to determine sanity.
- **Loses only the civil liberty to "come and go"**
  - Patient does not have the right to leave.
  - Patient can "choose" to take his medication; however, he cannot "choose" to leave.

Table 12-1. Decision-Making Standards

	What It Means	Who Makes the Determination
Capacity	An assessment of your decision-making ability	Physician
Competence	A legal assessment of your ability to make medical decisions for yourself	Judge
Sanity	A verdict on your ability to make decisions and be held accountable for the consequences of those decisions	Jury

Assume the patient is competent unless you have clear behavioral evidence that indicates otherwise.

- Competence is a legal issue.
- We do not determine competence on a medical basis.
- From an ethical and legal standpoint, all adult patients are considered competent unless specifically proven otherwise.
- Only a court of law can make that assessment.

Any physician—not just a psychiatrist—can determine whether a patient has the capacity to understand the medical issues (and related treatment) pertaining to his condition. The physician is able to determine whether there is an organic delirium affecting the patient's capacity to understand, i.e., caused by a medical condition such as alcohol/drug intoxication, meningitis, or a psychiatric disorder. The conclusions made by the physician will be based primarily on a neurological exam, as well as an assessment of the patient's comprehension, memory, judgment, and reasoning skills.

It should be noted that a diagnosis such as schizophrenia by itself tells you little about a patient's competence. If the patient is diagnosed with schizophrenia and controlled on medication, the patient may very well be competent. So a diagnosis alone cannot render a patient incompetent.

Clear behavioral evidence of incompetence includes:

- An attempted suicide
- Gross impairment in reality (psychosis) and dysfunctionality **and/or** physical or mental state preventing patient from having simple conversation

Patients who attempt suicide, for example, may be admitted to the hospital against their will for psychiatric evaluation.

Note

A physician is permitted to "detain" a patient for up to 48 hours.

If, as the physician, you are unsure, you must assume the patient is competent. The patient does *not* have to prove to you that he is competent. There must be clear evidence to assume that he is not.

When patients are unable to make medical decisions for themselves, someone called a **surrogate** needs to make those decisions for them.

In order for a surrogate to make a decision, three conditions must be present:

- The patient must be **incapacitated**.
- The patient must **not have made an advance directive**.
- The surrogate must know **what the patient would truly want if he were competent**.

Suppose a woman is unconscious as a result of a severe car accident. The surrogate will be asked, "What do you think the patient would want if she were conscious?" Based on the response, the patient will be appropriately treated or treatment measures will be withdrawn.

When a surrogate makes a decision for a patient, use the following criteria and **in this order**:

1. Subjective standard
2. Substituted judgment
3. Best interests standard

There is a set priority, i.e., an order, of who can serve as a surrogate. First is a person's spouse. Second is a person's adult children:

1. Spouse
2. Adult children
3. Parents
4. Adult siblings
5. Other relatives

Suppose the patient mentioned earlier is determined to be "brain dead" as a result of the car accident, and it is determined that she had no advance directive. Her spouse would be the first person asked about potentially terminating life support and allowing nature to take its course.

A **subjective standard** is based on the premise that a decision is being made based on the actual wishes of the patient. You should consider the following questions:

- Is there an actual intent or advance directive?
- What did the patient say in the past? Can that be verified?

Always follow the advance directives outlined in a living will or by a Health Care Power of Attorney (HCPOA).

A **substituted judgment** begins with the premise that decisions belong to the competent patient by virtue of the rights of autonomy and privacy. You should consider the following questions:

- Who best represents the patient?
- What would the patient say if she were competent?

In both ethical and legal terms, the patient has the right to make medical decisions but is now unable to do so by virtue of incompetence. The key here is not to identify the closest next of kin, but to identify the person most likely to represent the patient's own wishes, i.e., the person who knows the patient best.

For a **best interest standard**, the primary objective is to decide what action a hypothetical "reasonable person" would take after weighing the benefits and burdens of a particular medical decision or course of action. The issue of who actually makes the decision is less important, as all those applying the principles of best interest standard should come to the same conclusion. You should consider the following question:

- Are these in the best interest of the patient and not in the best interest of the decision-maker?

Note

An advance directive can be given in writing or given orally.

Advance Directives

An advance directive is a set of instructions given by a patient in anticipation of the need for a medical decision in the event the patient becomes incompetent. There are three primary forms of advance directive:

- An **oral advance directive** includes statements made by a patient prior to incapacitation.
  - Problems can arise from variance and interpretation; e.g., was the patient properly informed before she became incapacitated? How specific was the directive?
  - Good rule to follow: the more people who heard the oral directive, the more valid the directive.
- A **living will** is a written advance directive detailing the treatment measures the patient would want to receive (or not receive) should decision-making capacity be lost.
  - A family member cannot override patient's wishes; i.e., if the patient's living will indicates she does not want to be intubated in the event she becomes incapacitated but the patient's family requests intubation, the physician cannot intubate.
- A **medical power of attorney** or HCPOA is a designated agent assigned by the patient to make medical decisions in the event she loses decision-making capacity.
  - Assumption is that the agent fully understands the wishes of the patient and essentially "speaks with the patient's voice."
  - The physician must follow the directives of this individual, irrespective of other family members' wishes.

Do not resuscitate (DNR) orders are made by the patient or the surrogate. DNR refers **only to cardiopulmonary resuscitation**.

- In many instances, the physician may not be aware of DNR decisions.
- If DNR order is in place, cardiopulmonary resuscitation measures must be stopped as soon as the physician becomes aware of the order.
- All other treatment measures should be continued.

Patient Confidentiality

Patient confidentiality is absolute. Physicians require patients to divulge private information, and in doing so are required to keep all discussions confidential. Breach of trust can cause irreparable harm to the physician-patient relationship.

- **Physicians must strive to ensure that others cannot access patient information.**
  - Patient care must not be discussed with another health care provider in a public venue, where others can overhear the conversation (lunch room, elevator).
  - Patient's physical and electronic medical records must be protected.
  - Health care provider owns the medical records, but patient must be given access or copy upon request.
- If you receive a court subpoena, show up in court but do not divulge information about your patient. When asked personal health information questions about a patient, you should maintain patient confidentiality.

There a few **exceptions to patient confidentiality**.

- **Duty to warn and to protect** (Tarasoff case)
  - If patient is a threat to self or others, the physician *must* break confidentiality.
  - There is a duty (on the part of the physician) to warn and protect innocent people from harm that could be imposed by "your patient."
- Specific threat to a specific person
- Suicide, homicide, and child/elder abuse
- Infectious diseases may need to be reported to public officials or an innocent third party
- Impaired drivers

Note

The Health Insurance Portability and Accountability Act (HIPAA) protects the privacy of individually identifiable health information. HIPAA violations can result in imprisonment and substantial fines.

Table 12-2. Rules of Privacy in Health Care

Situation	Appropriate Response
Questions from insurance company	Obtain a release from patient
Questions from patient's family	Requires explicit permission from patient
When to withhold information from patient	Never, i.e., under no circumstances (if concerned about negative reaction by patient, figure out a way to explain and mitigate negative outcome)

Treatment of Minors

Children age <18 years are minors and thus legally incompetent. Children cannot make health-related decisions for themselves, thus they cannot give "informed consent" to authorize a medical procedure/treatment. A parent or guardian must provide informed consent instead.

Emancipated minors, however, present some exceptions:

- If child is age >13 and taking care of self (i.e., living alone, responsible for all aspects of own life), he is essentially treated as an adult.
- Person age <18 who is married
  - Child who marries age <18 is deemed competent to make own decisions.
  - Pregnancy or birth does not always emancipate; differences from state to state make this unlikely to be tested on the exam.
- Person age <18 serving in the military

Partial emancipation is granted to minors in some cases:

- Substance and drug-abuse treatment
- Prenatal care
- Sexually transmitted diseases
- Birth control

For example, a 15-year-old girl could go to a physician's office for evaluation of an STD or to request birth control, and the physician must treat her. Furthermore, the physician must respect her **confidentiality** pertaining to these issues.

Note

A child who comes home from school and takes care of brothers and sisters while waiting for mother and/or father to return from work would not be considered an emancipated minor.

Note

In general, the exam will not test controversial topics where there may be different state laws.

Withholding Treatment from Minors

Parents cannot withhold life- or limb-saving treatment from their children. If parents refuse permission to treat their child, then do the following:

- **Immediate emergency**: go ahead and treat
- **Not emergency but still critical** (e.g., juvenile diabetes): the child will be declared a ward of the court and the court grants permission
- **Not life- or limb-threatening** (e.g., minor stitches needed): listen to parents

Look at the following scenarios. For each, see who would provide consent.

Situation	Who Provides Consent
A 17-year-old girl's parents are out of the country and the girl is staying with a babysitter	If a threat to health, the physician can treat under doctrine of *in locum parentis*
A 17-year-old girl who has been living on her own and taking care of herself	The girl herself
A 17-year-old girl who is married	The girl herself
A 16-year-old daughter refuses medication but her mother consents	The mother; write the prescription
The 16-year-old daughter consents to medication, but the mother refuses	The mother; do not write the prescription
The mother of a minor consents to medication but the father refuses	Consent from only 1 parent is required; write the prescription

Physician-Assisted Suicide

Assisted suicide is suicide committed by a person with the assistance of another person.

- You cannot assist patients to commit suicide. You can permit "competent" patients to die by withdrawing medical treatment (feeding tube, hydration) at their request. However, you cannot provide patients with the means to commit suicide themselves.
- Well-informed and competent patients have an almost absolute right to refuse any part (including the whole) of a medical treatment. Even though you may not understand the reasoning behind the decision, you must respect patients' wishes.

Good Samaritan Laws

Good Samaritan laws limit liability when physicians help in nonmedical settings. Suppose a physician is driving down a road when she comes upon a three-car accident. No one else has stopped to help. She now has a choice to make: should she stop or not? As a physician she is not required to stop; i.e., if she does not, no civil or criminal charges would be brought against her.

The motorist who has crashed in this scenario is a "person," and not a "patient." Physicians are not required to stop and help at the scene of an emergency. If they do stop, they are acting as Good Samaritans. As such, the Good Samaritan laws limit a physician's liability, so long as certain conditions are met.

- **Actions are within the physician's competence.**
  - For example, every physician has been trained to administer CPR. If you have a medical bag with you, you could potentially administer some stitches. However, if the motorist was impaled with a large piece of wood, it is not likely you would be able to attend to the injury with your bag of medical supplies. Your focus would be to assess vital signs and keep the person calm while waiting for emergency services.

- **Only accepted procedures are performed.**
  - You would perform only standard of care procedures, not procedures that were considered alternative care or experimental.
- **The physician remains at the scene after starting therapy, until relieved by competent personnel.**
  - If you stop to assist a person, you need to stay there and provide assistance until the paramedics arrive.
  - In most instances, after receiving a call from 911 the paramedics are at the site of the action within a few minutes. Once they arrive and acknowledge that they now have the situation “under control” you can leave. However, you cannot treat the “person” and leave; you need to wait.
  - Preferably, join the ambulance and hand over the patient to another physician yourself.
- **No compensation changes hands.**
  - The person may offer you money or a gift as a “thank you” for stopping. You cannot accept any form of compensation.
  - If you accept compensation (or even a token gift), you become liable for the care provided because your services as a physician have been employed and the “person” now becomes your “patient.”

Negligence

Negligence is an act or omission (failure to act) by a physician or medical professional that deviates from the accepted medical standard of care. Negligence is often used regarding a civic duty. It can be intentional or unintentional:

- **Intentional**: malpractice
- **Unintentional**: medical negligence

Impaired Physician

Remove from patient contact health care professionals who pose risk to patients. Types of risks include:

- Infectious disease (TB)
- Substance related disorders
- Depression (or other psychological issues)
- Incompetence

Insist that they take time off; contact their supervisors if necessary. The patient, not professional solidarity, comes first.

Abuse

Abuse is defined by tissue damage, neglect, sexual exploitation, and mental cruelty.

Child abuse is a mandatory reportable offense up to age 18. Failure to report is a criminal offense.

If a case is reported in error, you (the physician) are protected from legal liability. Your duty to protect the child comes first. First separate child from the parents (protect); then report.

- Most cases of physical abuse involve injury to soft tissues (bruises, burns, and lacerations); in some cases there are no signs at all.
- Clinical signs suggesting abuse of a child include broken bones in year one of life, STD, and bruises, burns, and lacerations with incongruent explanations.
- Pay attention to injury location: soft tissue injury of inaccessible parts of the extremities/trunk.
- Non-accidental burns have a particularly poor prognosis.
- Shaken baby syndrome: look for broken blood vessels in eyes.

Note

Be careful not to mistake benign cultural practices, such as coining or moxibustion, for child abuse, but treat female circumcision as abuse.

Table 12-3. Types of Abuse

	Child Abuse	Elder Abuse	Domestic Abuse
Annual cases	>2 million	5–10% in population	>4 million
Most common type	Physical battery/neglect	Neglect (most common with 50% of all reported cases); physical, psychological, or financial	Physical battery
Likely gender of victim	Age <5: female Age >5: male	63% female	Female
Likely gender of perpetrator	Female	• Male or female • Caretaker is most likely source of abuse; spouses are often caretakers	Male
Mandatory reportable?	Yes	Yes, mandatory reportable offense	No, not mandatory reportable offense
Physician's response	Protect and report	Protect and report	• Counseling and information • Provide the victim information about local shelters and counseling; abused spouses tend to identify with the aggressor and blame themselves for the abuse
Comments	**Child sexual abuse** is defined as sex experienced age <18 with someone 5 years older: • >25% of adult women report being sexually abused as a child • 50% of perpetrators are family members • 50% of victims tell no one		

PHYSICIAN BEHAVIOR

The primary reason for **rejection of medical advice**, **changing physicians**, and **missed appointments** is a lack of rapport between the patient and physician. Failure of a patient to cooperate or even to keep appointments should be seen as the result of physician insensitivity or seeming indifference.

The key is not the amount of time spent with a patient, but what is done during that time. A good rapport fosters adherence to treatment regimens and is positively associated with a reduction of malpractice suits.

Patient-Centered Interview

Nothing should be between you and your patient.

- Get rid of tables and computers.
- Ask family members to leave the room, unless patient requests unprompted that they stay.
- Ask questions focused on the patient's feelings and needs.
- Address patient's concerns.

Anything that increases communication is good.

- Take the time to talk with patient, even if others are waiting.
- Ask follow-up questions for clarification and try to understand patient's thinking; ask "why"; ask about personal issues beyond the disease: job, family, children.
- Be available (take calls and answer emails).
- Respond to the emotional as well as the factual content of questions from your patient.

Tell the patient everything, even if you have not been asked.

- Answer any question that is asked of you; if you only have partial information, let the patient know and provide the information you do have.
- Do not force a patient to hear bad news if he resists, but inquire why so you can address the underlying fears as soon as possible.

Keep everything confidential.

- Information should flow through the patient to the family, not the reverse.

Consider the patient interview and history-taking an opportunity to develop a better relationship.

- Make eye contact; sit so you are at same eye level if possible.
- Work on a long-term relationship, not just short-term problems.
- Tell patient what you are doing before every physical maneuver (defined touch).

Listen more; talk less.

- Hearing from patient is most important; allow patient to choose words to describe (do not ask leading questions).
- Allow silences while patients search for words.
- Listen for clues and pay attention to body language; you know what matters but patient won't.
- Ask what patient knows before explaining.
- Provide opportunity for patient to ask questions of you.

Negotiate rather than order.

- Medical decisions are made by patient; the physician provides and explains the options.
- Treatment choices are the result of agreement.
- An agreement fosters adherence while instructions and commands do not.

Agree on the problem before moving to a solution.

- Informed consent requires the patient to fully understand what is wrong. Offering a correct treatment before the patient understands his condition is not the right approach.
- A patient may not articulate his problem clearly and might exhibit emotions without articulating an underlying problem at all.
  - Ask questions to get details first before you offer solutions.
  - Begin with open-ended questions, then move to closed-ended questions.
- Your patient's problem is your problem.
  - Talk and think of solutions, not "an answer."
  - Change your plan to deal with new information when it is presented.

Practice effective interview techniques.

- Open-ended questions allow broad range for answer; closed-ended questions limits answer to yes or no.
- Leading questions suggest a preferred answer.
- Direct questions seek information directly; avoid judgmental terms.
- **Confrontation** brings to the patient's attention some aspect of appearance or demeanor.
- **Facilitation** gets the patient to continue a thought, talk more, "tell me about that . . ."
- **Redirection** puts question back to the patient.

Physician-Patient Relationship

The physician-patient relationship is a partnership based on trust. In the setting of a productive alliance there are tremendous opportunities for clinical interventions that can significantly improve the patient's health and quality of life. The key is what the ideal physician should do to build rapport, establish trust, and maintain trust.

Keep the physician–patient relationship within bounds. Intimate social contact with anyone who is or has been a patient is prohibited. AMA guidelines recommend no interpersonal relationship with a former patient "for at least two years."

- Do not date parents of pediatric patients or children of geriatric patients.
- Do not treat friends or family.
- Do not prescribe treatment for colleagues unless a physician/patient relationship exists.
- If patients are inappropriate, gently but clearly let them know what acceptable behavior would be.
- Decline any gift from a patient beyond a small token.
- Tell patients everything: as a physician you must always be honest and transparent; there should be no lies or omissions.
- Admit to mistakes.

Remember your duty to the patient. Always place the interests of the patient first. Choose the patient's comfort and safety over yours or anyone else's. The goal is to serve the patient, not to worry about your legal protection.

- Ask about and know the patient's wishes.
  - Make conversations positive.
  - Patient cannot select inappropriate treatments but you must discuss options that are available. Don't just say no to a patient's request.
  - If a patient asks for an inappropriate medication that she heard advertised, explain why it is not indicated.
- Never "pass off" your patient to someone else. Refer to a specialist when something is beyond your expertise. You provide instruction in aspects of care, e.g., nutrition, use of medications.
- Be an advocate for the patient and try to get the patient what they need.
- Never refuse to treat a patient because she can no longer pay.
- Do not be involved in counseling about organ donation for a patient in your care. This undermines your physician-patient relationship.

Foster patient adherence. It is not enough for you to provide counsel/treatment and leave adherence to the patient. You must present information in ways that will optimize patient adherence. For best adherence:

- Attend to the amount of information; explain its complexity.
- Note the patient's affective state.
- Explain why a particular treatment is being recommended.

- Stress the threat of non-adherence to health.
- Stress the effectiveness of the prescribed regimen; give instructions both orally and in writing.
- Arrange for periodic follow-up.
- Ask the patient to do less; a long list of instructions is detrimental to adherence.

In cases of non-adherence:

- Do not blame the patient.
- Check for patient dissatisfaction with the physician, misunderstood instructions, family interference, or inability to pay for medication.

Never abandon a patient. Suppose a patient frequently comes to your office to complain about the office staff and your services. He even calls you on your cell phone to express discontent. You may be tempted to just dismiss the patient and let another physician deal with him. From an ethical standpoint, that would be patient abandonment.

- Make every effort to determine why the patient feels a certain way and then work to remedy the situation. Perhaps he has a psychiatric disorder that needs to be addressed.
- Never stop treatment on a patient due to lack of financial resources or treatment results. If a patient comes in with a medical complaint and he is past due on his medical bills, provide him with the same level of care and respect you would provide for any other patient.
- Do not ever threaten abandonment. If a patient is annoying or disruptive, you cannot say, "If you do not change your behavior, I will be forced to dismiss you from my practice and you will need to seek medical attention elsewhere."

Have empathy. Empathy is the capacity to place oneself in another's position. Empathy both acknowledges and validates a patient's feelings. Do not confuse it with sympathy, which is simply feeling bad for another person's suffering.

- Empathy: "I feel your pain. What can I do to help?"
- Sympathy: "I'm sorry for your pain."

Note

Treat all patients with the same level of care and respect, even if they do not reciprocate the same to you and your staff.

Accommodate different cultures. Offer language assistance services (translator) to patients with limited English proficiency (a legal right). It will help to facilitate proper communication.

- Interpreters: spoken communication
- Translators: written communication

Respect patients' religious beliefs, even when you do not share them. The goal is to make the patient comfortable.

- Ask about patient's beliefs.
- Accommodate religious practices: participate when requested and if possible (although you are not required to do anything against your own religious/moral beliefs or anything that risks the patient's health).
  - Suppose a patient requests you take an unsterile totem made of animal bone and bring it into surgery with you for good luck. You

might accommodate the request by placing the item into a sealed plastic pouch and take it with you while keeping it far from the surgical field.

  - Accept benign "folk medicine" practices. Expect them.
    - Moxibustion: dried plants called moxa are burned on or near the body.
    - Coining: a form of dermabrasion therapy in which a heated coin is dragged along the skin.
    - Cupping: inverted cups are placed on the skin and suction is applied either by heat or vacuum; leaves circular marks.
    - Acupuncture: Thin needles are inserted into the body; due to the potential for medical complications and ramifications (such as contaminated or infected needles), this is not a benign practice.
  - Explain medical diagnosis in a manner that can be understood by a patient, even if it is not technically precise.
  - Offer to facilitate discussions or explain things to family members.

Deal with difficult patients. Treat difficult or suspicious patients in a friendly, open manner. An annoying or difficult patient is still your patient. You cannot ever threaten abandonment.

Treatment Issues

- Suppose a patient's course of action is against your medical recommendation (a common scenario). Remember, any medical treatment can be withdrawn at the patient's request.
  - A feeding tube is a medical treatment and can be withdrawn at the patient's request.
  - A competent person can refuse even lifesaving hydration and nutrition.
  - You are not obligated to provide medical treatment that is inappropriate; in fact it is your duty to refuse to provide such care even when the patient demands it.
  - In other words; a patient can refuse care, but cannot demand inappropriate care.
- You cannot ethically act to facilitate a patient's death, but you may administer care to provide relief to a terminal patient, even if such care may hasten demise (providing pain medication for example).
  - Passive: allowing patient to die
  - Active: killing patient
  - Active euthanasia: administering lethal medication with the purpose of ending life (illegal in the United States)
  - Assisted suicide: providing a patient the means with which to take his own life (legal in some states in the United States)

- You decide when the patient is dead.
  - You are obligated to continue treatment at the behest of patients and their surrogate, even if they surmise from a patient's lack of improvement that such treatment is futile.
  - If you determine that the patient is dead, then treatment must stop.
  - Any physician can decide/declare a time of death for a patient and the courts (or a judge) will officially declare a patient to be dead. For example, if a patient is brought to the emergency department following a severe car accident and "dies" in the emergency room, the physician will "decide" the actual time of death. The physician will fill out the necessary paperwork and a judge will "declare" that the patient is dead.

Angry Patients

An irate patient requires care just as much a pleasant patient. The rule of thumb to **diffuse the situation**.

- Validate your patient's feelings and find ways to hand him back control of the situation: "If I had to walk up four flights of stairs with my leg in a cast, I'd be upset. Now that you are here, what would you like to do?"

Reluctant Patients

When a patient seems hesitant to share information, an assurance of confidentiality might be helpful.

- Silence is effective. A few moments of silence might be uncomfortable, but it allows the patient an opportunity to collect her thoughts and reinforces that you are there to listen to her problem.

Sick role

The sick role is a "limited and conditional" set of expectations that are attached to individual persons socially when they are defined as being "sick." These expectations are held dependent on both the nature and severity of the condition.

Rights

- Person is exempt from normal responsibilities: can stay home from work and not do chores around the house.
- Person is not to blame for illness: anyone can get the flu and there is no "fault."

Obligations

- Person is obligated to get well: should rest, drink fluids, try to eat.
- Person is obligated to seek competent help: for the flu a patient may not be required to go to the doctor; however, some employers require a "doctor's note" upon return to work.

UNCONSCIOUS INTERACTIONS

Patients and physicians may unconsciously react to each other. These are not defense mechanisms, although they may seem to function similarly. These reactions can be classified as transference and countertransference.

Transference: patient may unconsciously transfer thoughts onto physician:

- Unconscious
- Thoughts or attitudes are typically of a parent or significant other
- Patient identifies within the physician similar traits that lead the association and transference
- Transference may be positive (cause you to unaccountably like someone) or negative (cause you to unaccountably dislike someone)
- Likelihood of transference is not related to the duration of treatment.

Countertransference: physician may unconsciously transfer thoughts onto patient:

- Affects attitude of the physician toward patient
- May be positive (physician wants to help an elderly patient because she reminds him of his parent)
- May be negative (physician unaccountably dislikes a patient)

Practice Questions

For the scenarios below, identify whether you would treat or not treat.

1. A patient refuses lifesaving treatment on religious grounds
2. A wife refuses to consent to emergency lifesaving treatment for her unconscious husband, citing religious grounds
3. A wife produces a card stating her unconscious husband's wish to not be treated on religious grounds
4. A mother refuses to consent to emergency lifesaving treatment for her daughter on religious grounds
5. A child's life is at risk, but the risk is not immediate

Answers

1. Do not treat.
2. Treat the woman's husband; this is no time to assess substituted judgment.
3. Do not treat the woman's husband.
4. Treat the woman's daughter.
5. Court will take guardianship.

Health Care Delivery Systems 13

Learning Objectives

- ❑ Answer questions about the different types of payer systems
- ❑ Demonstrate understanding of the basic definitions of health care

PAYER SYSTEMS

	Definition	Characteristics
Private Insurance	Medical plan that patients purchase to hedge against medical costs	• Patient pays monthly premium • When/if patient is ill, insurance company will pay for bulk of the medical bills
Medicare	Federal government program that makes health care payments to those on Social Security	• Program pays health care costs for elderly (age >65), disabled, and dependents of disabled • Part A pays for hospital care • Part B pays for physician services • Annual deductibles and copayments are applicable
Medicaid	Joint state/federal program that covers all care for those on welfare	• Covers hospital stays, physician services, medication, and nursing homes • There are no deductibles or copayments
Health Maintenance Organization (HMO)	Prepaid group practice that hires physicians or contracts with physicians to provide services	• Payment is made by capitation: fixed payment for the number of patients in their care • Physicians receive only minor additional compensation for care when it is provided • Preventive care is incentivized
Preferred provider organization (PPO)	Fee-for-service at a discount	• Provider makes money on volume, i.e., less money per patient but more patients • Efficiency is rewarded

DEFINITIONS

Deductible

Before insurance assistance begins, patients must pay a certain amount called a deductible. After the detectible is "met," the remainder of the bill is divided between the patient and insurance company (**co-insurance**).

- In an annual deductible, patient pays certain amount each year
- In a per-occurrence deductible, patient pays certain amount each time services are rendered
- **Copayment** is a flat fee due at time of service that is based on type and location of service (e.g., primary care $25, specialist $45)
- **Coinsurance** is the portion, or percentage, of final bill that patient and insurance are each responsible for paying (e.g., 80/20 = insurance covers 80% of remaining bill and patient is responsible for 20%) (full coverage means insurance covers 100% of bill)

Capitation

Capitation is a fixed, pre-arranged monthly payment made for each patient.

- Physicians are paid for number of patients they are responsible for, not for how "much" they do for each patient.
- Same payment is made whether services are used or not.
- No additional (or only minimal) payment is made when services are used.
- Physicians make money when patients stay well and require no services.
- Under-treatment is incentivized, but also more likely to foster preventive medicine.

Catastrophic Coverage

Catastrophic coverage is insurance for big medical events.

- It is more appealing for younger patients who do not expect to have medical expenses.
- Insurance premiums are lower, but out-of-pocket costs are larger if one becomes sick.

Medically Indigent Adults

Medically indigent adults (MIAs) do not have private health insurance. They are not eligible for other health care coverage, such as Medicaid or Medicare.

PART III

Social Sciences

Basic Science of Patient Safety

14

Learning Objectives

- ❑ Answer questions about scope of patient safety problems
- ❑ Describe the categories of medical error
- ❑ Answer questions about the systems approach to medical error and failure analysis
- ❑ Analyze cases concerning error disclosure and reporting
- ❑ Demonstrate understanding of principles of quality improvement
- ❑ Explain the leadership role of physician to lead change in patient safety

INTRODUCTION

Case 1: Care done well

A 3-year-old girl falls into an icy fishpond in a small Austrian town in the Alps. She is lost beneath the surface for 30 minutes before her parents find her on the pond bottom and pull her up. CPR is started immediately by the parents on instruction from an emergency physician on the phone, and EMS arrives within 8 minutes. The girl has a body temperature of 19 C and no pulse. Her pupils are dilated and do not react to light. A helicopter takes the patient to a nearby hospital where she is wheeled directly to an operating room. A surgical team puts her on a heart-lung bypass machine, her body temperature increases nearly 10 degrees, and her heart begins to beat. She requires placement on extracorporeal membrane oxygenation. Over the next few days her body temperature continues to rise to normal, and the organs start to recover. She suffered extensive neurologic deficits; however, by age 5, after extensive outpatient therapy, she recovers completely and is like any other little girl again.

Case 2: Failure of the medical system

A newborn baby boy is first noted to be jaundiced through visual assessment hours after delivery, but a bilirubin test is not done. At the time of discharge from the hospital, the child is described as having "head to toe jaundice," but a bilirubin test had still not been done, nor had his blood type or Coombs test been performed. The parents are instructed that the jaundice is normal and they should not worry, and to simply place the infant in the window for sunlight. A few days later the baby's mother calls the newborn nursery stating that her son is still yellow,

lethargic, and feeding poorly. She is asked if she is a "first-time mom" and then assured that there was no concern. The mother continues to notice that the child is not well. At age 5 days, the mother's concerns are acknowledged and a pediatrician admits the baby boy to the pediatric unit. On day 6 in the afternoon, the child has a high pitched cry, respiratory distress, and increased tone. He also starts to arch his neck in a way that is characteristic of opisthotonos. The child is ultimately diagnosed with classic textbook kernicterus, resulting in permanent brain damage.

The 2 real cases above represent the reality of our current health care system and the issues of patient safety. In one case a series of complex processes result in an excellent outcome, while in another a patient suffers preventable injury.

What are the factors that cause a good versus poor outcome? The field of patient safety seeks to answer this question and take steps to prevent future patients from being harmed by medical errors.

Patients are at risk for sustaining harm from the health care system and do so at an alarmingly high rate. Injury can range from minor to severe incidents, including death. The cause of these adverse events is not usually intentional injury (i.e., someone intending to harm patients), but rather is due to the complexity of the health care system combined with the inherent capability of human error.

The prevalence of medical errors in the United States is a significant and ongoing problem. Media reports of catastrophic injury resulting in disability or death due to medical care often reach news headlines, and are a significant concern to patients, families, and members of the health care team. The causes of these errors are varied, and can include failures in the administration of medication, performing surgery, reporting laboratory results, and diagnosing patients, to name a few.

Ensuring patient safety is the responsibility of every member of the health care team. To do so requires an understanding of safety science and quality improvement principles. Patients, providers, payers, and employers are all stakeholders in improving patient safety. Applying these principles to the study of medical errors can help health care professionals learn from past errors and develop systems that prevent future errors from harming subsequent patients. Systems in health care delivery can be redesigned to create safeguards and safety nets which make it difficult for members of the health care team to make errors that harm patients. The goal of health care should be to learn the strategies and systems that are currently being put into place to improve patient safety.

SCOPE OF THE PROBLEM

In 1999 the Institute of Medicine (IOM) published its landmark publication, "To Err is Human: Building a Safer Health System," reporting that at least 44,000 people—and perhaps as many as 98,000—die in hospitals each year as a result of medical errors that could have been prevented. This exceeds deaths attributed to breast cancer, motor vehicle collisions, and HIV. Approximately 1 in 10 patients entering the hospital will suffer harm from an adverse event.

Patient harm from preventable medical errors is a serious concern in health care. The impact of these errors can have dramatically negative effects on patients, their families, and the health care personnel involved. In addition to the

toll on human suffering, medical errors also present a significant source of inefficiency and increased cost in the health care system.

Medical errors are the eighth leading cause of wrongful death in the United States. The problem is not limited to this country, however; medical errors are a global problem.

Some of the more common contributors to medical errors and adverse patient events are as follows:

Medication errors represent one of the most common causes of preventable patient harm. An estimated 1.5 million deaths occur each year in the United States due to medication error. The IOM estimates that 1 medication error occurs per hospitalized patient each day.

Common causes of medication error:

- Poor handwriting technique on a prescription pad or order form, resulting in a pharmacist or nurse administering the wrong drug or wrong dose
- Dosing or route of administration errors
- Failure to identify that given patient is allergic to a prescribed medication
- Look-alike or sound-alike drugs (e.g., rifampin/rifaximin)

Figure 14-1. "Look-Alike" Medications

Strategies that help to reduce or prevent medication errors are as follows.

- The **5 Rs** help to confirm several key points before the administration of any medication.
  - Right drug
  - Right patient
  - Right dose
  - Right route
  - Right time
- Computerized physician order entry (CPOE) involves entering medication orders directly into a computer system rather than on paper or verbally. The computer software (i.e., electronic health record) can automatically check for prescribing errors or allergies.

Hospital-acquired infections (HAI) affect 5–15% of all hospitalized patients and 40% of patients in ICU. The World Health Organization (WHO) estimates that the mortality from health-care-associated infections ranges from 12–80%.

HAI can occur in many forms, the most common of which in hospitalized patients is **urinary catheter-related infection** (UTI). UTI accounts for 40% of all HAI; >80% of these infections are attributable to use of an indwelling urethral catheter. Adhering to strict indications for using indwelling catheters, maintaining sterile technique during catheter insertion and exercising prompt removal of the catheter when it is no longer required can help reduce the risk of a urinary catheter-related infection.

Central line associated bloodstream infection (CLABSI) is another common HAI, and among one of the most common infections observed in patients admitted to critical care units. It is estimated that 70% of hospital-acquired bloodstream infections occur in patients with central venous catheters. Symptoms include fever, chills, erythema at the skin surrounding the central line site and, in severe cases, hypotension secondary to sepsis. These infections can be associated with significant morbidity and mortality, increased length of hospital stay, and increased hospital cost. Checklists have been developed which provide best practices for the placement of central lines that lower the risk of infection (e.g., hand washing, gloving and gowning, sterile barriers, and early removal of central lines when possible).

Hospital-acquired pneumonia (HAP) is an infection that occurs more often in ventilated patients, typically ≥48 hours after admission to a hospital. These ventilator-associated pneumonias (VAP), a subtype of HAP, tend to be more serious because patients are often sicker and less able to mount effective immune responses. HAP is the second most common nosocomial infection. Common symptoms include coughing, fever, chills, fatigue, malaise, headache, loss of appetite, nausea and vomiting, shortness of breath, and sharp or stabbing chest pain that gets worse with deep breathing or coughing. Several methods have been undertaken to prevent HAP, including infection control (e.g., hand hygiene and proper use of gloves, gown, and mask), elevation of the head of the bed in ventilated patients, and other measures to reduce the risk of aspiration.

Surgical site infections (SSI) occur following a surgical procedure in the part of the body where the surgery took place. Some SSIs are superficial and limited to the skin, while others are more serious and involve deep tissue under the skin, body cavities, internal organs, or implanted material (e.g., knee or hip replacements). Symptoms include fever, drainage of cloudy fluid from the surgical incision or erythema, and tenderness at the surgical site. Most superficial SSIs (e.g., cellulitis) can be treated with appropriate antibiotics, whereas deeper infections (i.e., abscess) require drainage. Pre-operative antibiotics have been effective in reducing the rate of SSIs.

Patient falls are a common cause of injury in hospitals and other health care settings such as nursing homes. Over 1/3 of elderly people age >65 fall each year. Researchers estimate that >500,000 falls happen each year in U.S. hospitals, resulting in 150,000 injuries. Approximately 30% of inpatient falls

result in injury, with 4–6% resulting in serious injury. Injuries can include bone fractures, head injury, bleeding, and even death. Injuries from falls also increase hospital costs.

Assessing a patient's fall risk helps to identify high-risk patients who can benefit from preventative resources. Some risk factors include advanced age (age >60), muscle weakness, taking >4 prescription medications (especially sedatives, hypnotics, antidepressants, or benzodiazepines), impaired memory, and difficulty walking (e.g., use of a cane or walker). Interventions such as increased observation, nonslip footwear, and making the environment safe all play a role in preventing injury from falls.

Unplanned readmissions occur when patients unexpectedly return to the hospital <30 days after being discharged. According to a *New England Journal of Medicine* study analyzing close to 12 million Medicare beneficiaries, nearly 20% of those discharged were readmitted within 30 days. Several factors can lead to a hospital readmission, such as poor quality of care or breakdowns in communication during a transition of care (e.g., hospital to rehabilitation center). Readmissions may also occur if patients are discharged from hospitals prematurely, are discharged to inappropriate settings, or if they do not receive adequate information or resources to aid in recovery.

For example, a 79-year-old patient treated for congestive heart failure (CHF) returns to the hospital 10 days after discharge with exacerbation of CHF. It was discovered that upon release the patient had failed to fill the prescription for the diuretic started during the initial hospitalization. Improved communication, patient education, and increased support to patients at risk for readmission are all strategies to reduce unplanned readmissions.

CAUSES OF MEDICAL ERROR

The miraculous recovery of the little girl after the drowning event in case 1 highlights the incredible complexity of our modern health delivery system. There were numerous steps that were required to get right in the care of the patient. Unfortunately, these steps are not always followed as well as they were in that case. Machines break down, a team can't get moving fast enough, or a simple step is forgotten or the wrong step applied. The greater the number of steps required, the greater the risk of something going wrong. Couple that with the fact that human beings are prone to error, especially when working under less-than-ideal circumstances, and it is no wonder that medical errors pose such a threat to health care.

The complexity of the health care system, together with the potential for mistakes due to human nature, is the primary reason that patients experience medical errors. Understanding medical error and the science of patient safety can help us design a health care system capable of getting it right every time.

The potential for human error is amplified by poor working conditions. This includes poor workplace conditions (e.g., overworked staff, time pressures, lack of safety protocols, or lack of appropriate supervision), as well as poor individual conditions (e.g., fatigue, stress, or illness).

Note

Errors are bound to occur due to a combination of a complex health care system and the reality of human fallibility.

The following is a mnemonic to help assess the fitness of a health care professional to attend to patient care.

IM SAFE

- **I**: Illness (Are you suffering from an illness that is degrading your performance?)
- **M**: Medications (Are you taking medications that may impair your judgment?)
- **S**: Stress (Are you adequately managing the stressors in your life?)
- **A**: Alcohol (Are you using alcohol in excess with negative consequences?)
- **F**: Fatigue (Are you getting enough rest?)
- **E**: Eating (Are you maintaining a healthy diet?)

For example, one study on physician performance found that being awake 24 hours was equivalent to having a blood alcohol level of .10 (legally intoxicated by most standards) (Dawson & Reid, 1997).

Communication and teamwork failures are another leading cause of adverse patient events. Lack of appropriate communication creates situations where medical errors are likely to occur. These errors have the potential to cause severe injury and unexpected patient death. Errors at the time of transitions or handoffs are among the most common communication errors in healthcare. Handoffs occur frequently between nurses and between residents in teaching hospitals, but also among attending faculty (e.g., on-call physicians, hospitalists, ED staff). Using techniques of structured communication (e.g., SBAR, call-backs) can help safeguard against errors. Poor teamwork and lack of coordination between members of the patient care team also result in medical errors. A growing recognition of the need for improved teamwork in health care has led to the application of teamwork training principles, originally developed in aviation, to a variety of clinical settings. Recognized barriers to effective teamwork include:

- Inconsistency in team membership
- Lack of time
- Lack of information sharing
- Hierarchy
- Defensiveness
- Conventional thinking
- Complacency
- Varying communication styles
- Conflict
- Lack of coordination
- Distractions
- Fatigue
- Workload
- Misinterpretation of cues
- Lack of role clarity

Teamwork training attempts to reduce the potential for patient harm by developing effective communication skills, a supportive working environment, and an atmosphere in which all team members feel comfortable speaking up when they suspect a problem. Team members are trained to cross-monitor; check each other's actions, offer assistance when needed, and address errors in a nonjudgmental fashion (i.e., watch each other's backs). Huddles, briefs and debriefs are essential components of teamwork training as is providing feedback, especially after critical incidents. Remember, a chain is only as strong as its weakest link.

TYPES OF MEDICAL ERROR

Types of Errors

Diagnostic

- Error or delay in diagnosis
- Failure to employ indicated tests
- Use of outmoded tests or therapy
- Failure to act on results of monitoring or testing

Treatment

- Error in the performance of an operation, procedure, or test
- Error in administering the treatment
- Error in the dose or method of using a drug
- Avoidable delay in treatment or in responding to an abnormal test
- Inappropriate (not indicated) care

Preventive

- Failure to provide prophylactic treatment
- Inadequate monitoring or follow-up of treatment
- Other
  - Failure of communication
  - Equipment failure
  - Other system failure

Source: Leape, Lucian; Lawthers, Ann G.; Brennan, Troyen A., et al. Preventing Medical Injury. Qual Rev Bull. 19(5): 144–149, 1993

Errors can be categorized as slips, lapses, or mistakes.

- **Slips** can be thought of as actions not carried out as intended or planned, e.g., injecting a medication intravenously when you meant to give it subcutaneously. Slips are observable.
- **Lapses** are missed actions and omissions (e.g., forgetting to monitor and replace serum potassium in a patient treated with furosemide for acute congestive heart failure). Lapses are generally not observable (i.e., one cannot directly "see" a lapse of memory).

Both slips and lapses are actions that do not "go as intended."

- **Mistakes** are a specific type of error brought about by a faulty plan or incorrect intentions; the intended action is wrong (e.g., extubating a patient prematurely based on misapplication of guidelines, or treating a patient for a suspected pneumonia when the patient was misdiagnosed and actually has a pulmonary embolism).

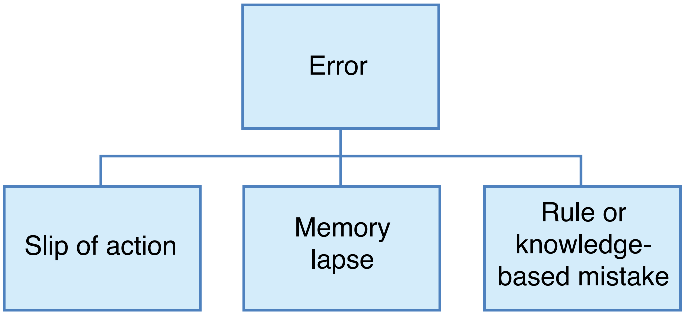


Figure 14-2. Types of Error

It is important to differentiate errors (slip, lapse, or mistake) from violations.

- **Violations** are deliberate actions, whereby someone does something and knows it to be against the rules (e.g., deliberately failing to follow proper procedures). A health care professional may consider that a violation is well-intentioned; however, it would still technically constitute a "violation" rather than an error. For example, a physician may decide to forgo entering a patient's allergies into the electronic record due to time constraints in starting treatment. If this act led to an adverse medication reaction due to a missed allergic reaction, it would technically be considered a violation and not an error.

Errors may result in adverse events or near-misses.

- **Adverse events** are harms or injuries that result directly from medical care, not from negative outcomes due to the patient's disease or medical condition.
- **Near-misses** are errors that occur but do not result in injury or harm to patients because they are caught in time or simply because of luck.

Diagnostic errors account for at least 17% of preventable errors in hospitalized patients. Diagnostic errors can be categorized as no-fault, system-related, and cognitive.

- **No-fault errors** may happen when there are masked or unusual symptoms of a disease, or when a patient has not fully cooperated in care.
- **System-related** errors include technical failure, equipment problems, and organizational flaws.
- **Cognitive errors** frequently result from a diagnosis that was wrong, missed, or unintentionally delayed due to clinician error.

The following are examples of common cognitive errors.

A wrong diagnosis may occur when the clinician holds on to a particular diagnosis (usually the initial one, in a phenomenon called **anchoring bias**) and becomes dismissive to signs and symptoms pointing to another diagnosis. For example, a 65-year-old man presents with epigastric pain and emesis, and he sits leaning forward. He has a history of alcoholism. The patient is likely to be diagnosed with pancreatitis. However, holding on to this diagnosis to the exclusion of any other diagnosis—despite the patient's denial of alcohol use for several years, normal blood levels of pancreatic enzymes, and abnormal EKG which is ignored—would be an anchoring error.

Confirmation bias, looking for evidence to support a pre-conceived opinion, rather than looking for evidence that refutes it or provides greater support to an alternative diagnosis, may accompany an anchoring error. Clinicians should regard conflicting data as evidence for the need to continue to seek the true diagnosis (e.g., in the case above; acute MI) rather than as anomalies to be disregarded.

Availability bias is the tendency to assume a diagnosis based on recent patient encounters or memorable cases (i.e., the most cognitively "available" diagnosis).

It is estimated that thousands of hospitalized patients die every year due to diagnostic errors. Missed or delayed diagnoses (particularly of cancer) are a prominent reason for malpractice claims. Poor teamwork/communication between clinicians and a lack of reliable systems for common outpatient clinical situations (e.g., triaging acutely ill patients by telephone and following up on test results) have been identified as predisposing factors for diagnostic error.

SYSTEMS APPROACH TO MEDICAL ERROR

Health professionals dedicate their lives to the care of patients. Most are highly trained and competent; however, the nature of health care is extremely complex, and people, despite good intentions, are still capable of making errors.

Although hospitals, clinics, and doctor's offices take many steps to keep their patients safe, medical errors can, and do, occur. Rather than penalize individuals who make honest mistakes, the goal of patient safety is to redesign systems to be more fool-proof and able to compensate for human error.

Bad Apples/Blame Culture: When a medical error occurs, the bad apple approach seeks to identify who is responsible for the error and take punitive action against that individual. However, this approach does not improve safety. It creates a culture of fear and doesn't address the root cause of the error.

Only 5% of patient harm is due directly to incompetence or poor intentions. People need to be accountable, but systems changes are needed to truly transform care. Unfortunately, health care has a long tradition of a blame culture. Blaming people who make errors does not get to underlying causes or help to prevent the error from happening to someone else in the future.

The most effective approach to reducing harm from medical error is to find out *how* the error happened, rather than who did it, and then fix the system to prevent errors from causing injury to patients. Improvements in patient safety will be hindered as long as there is a focus on blaming individuals.

SYSTEMS APPROACH TO FAILURE

An understanding of medical error requires an understanding of the systems failures underlying the majority of adverse patient events. Health care is a complex system. Errors that harm patients tend to have multiple causes that are ingrained in this complex system. James Reason, a pioneer and leader in the research area of human error and organizational processes, describes the **Swiss cheese model** of accident causation; it is a model used in risk analysis and risk management in complex systems including health care.

The Swiss cheese model encompasses the understanding that patient harm often results from multiple, upstream or proximal errors. In the Swiss cheese model, each 'slice' represents a barrier, and each hole is a failure in the system due to either active or latent failures. Under normal circumstances, one of the barriers works to prevent patient harm (e.g., the nurse catches that the medication ordered is the wrong dose before giving it to the patient); however, occasionally the perfect storm scenario arises where the holes line up and allow an error to reach the patient, resulting in harm.

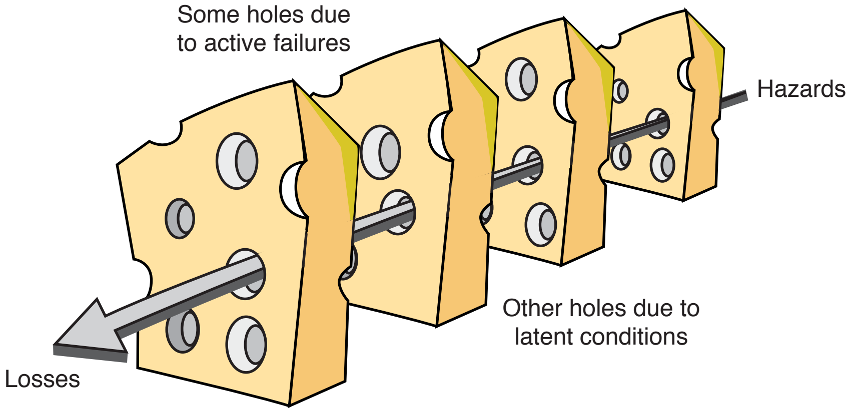
For example, if the hazard were wrong-site orthopedic surgery, slices of cheese might include policies for identifying sidedness on radiology imaging, a protocol for signing the correct site when the surgeon and patient meet in the preoperative area, and a second protocol for reviewing the medical record and checking the previously marked site in the operating room. Many more layers exist but the point is that no single barrier is foolproof. They each have "holes," hence, the Swiss cheese.

Note

Approximately 80% of medical errors or adverse patient events are system-derived.

In some serious events such as wrong site surgery, even though the holes will only align infrequently, the result is still unacceptable patient injury. For instance, in an emergency situation, all 3 of the surgical identification safety checks mentioned above may fail or be bypassed, resulting in the surgeon meeting the patient for the first time in the operating room already under anesthesia. A hurried x-ray technologist might mislabel a film (or simply hang it backward and a hurried surgeon may not notice), confirming the surgical site with the patient may not take place at all (e.g., if the patient is unconscious) or, if it takes place, be rushed and offer no real protection.

Under the blame culture traditionally present in health care, a person may be reprimanded for an error but the holes in the system are not addressed; making it quite probable that the same error will be committed by someone else in the future leading to more patient harm. The goal is to examine the system and develop methods to redesign care so that the holes are removed.



Source: James Reason

Figure 14-3. Successive Layers of Defenses, Barriers, and Safeguards

Other industries with complex systems, such as aviation and nuclear power plants, have successfully employed systems engineering to drastically improve safety and reliability. These industries have also made changes to improve communication, teamwork, and the culture of safety.

Another example of lessons learned from systems engineering is the automobile safety industry. Most motor vehicle collision (MVC) deaths are due to driver error or deliberate misbehavior (e.g., speeding, running a red light, failure to wear a seatbelt, etc.). The death toll from MVC in the past several decades has declined significantly. Drivers today are not necessarily safer drivers than before; however, design changes in cars (e.g., collapsible steering columns, airbags) and safe highway design (e.g., improved lighting, deformable lampposts) have resulted in drastic reductions in mortality from MVCs.

Likewise, the goal in patient safety is to prevent errors from resulting in harm to patients. Health care must create safety nets that absorb mistakes before they reach patients. Some examples of system-based redesigns for patient safety include protocols to ensure proper patient identification, such as the following:

- Using at least 2 methods such as patient name and date of birth to confirm patient identify prior to the administration of medications
- Using a standardized pre-operative checklist to help operating room staff review critical information prior to surgery (e.g., pre-operative antibiotics)
- Removing look-alike drugs from the nursing unit in order to prevent medication errors

ERROR DISCLOSURE AND REPORTING

Many victims of medical errors never learn that the mistake occurred, because the error is simply not disclosed. Healthcare professionals have traditionally shied away from discussing errors with patients, due to fear of precipitating a malpractice lawsuit, issues of professional embarrassment, or discomfort with the disclosure process. It is both an ethical and professional responsibility to ensure that errors resulting in patient harm are disclosed and reported.

The first priority after an event which causes patient harm is to care for the patient's medical needs. **Disclosure** of the error is another important early action. Following an adverse event, patients and families want to hear an apology and to know what is being done to prevent the error from harming someone else in the future. They may also require emotional and social support. Honesty and transparency are essential. There is no role for covering up an error (e.g., altering documentation to conceal the error) or withholding information from the patient or family. Such practices are unethical and betray the professional responsibility we have to patients. Studies have demonstrated that a timely and sincere apology may actually reduce the likelihood of a lawsuit.

Often the most senior physician responsible for the patient and most familiar with the case will make the official disclosure. An error disclosure should include the following 3 elements:

- Accurate description of the events and their impact on the patient
- Sincere apology showing care and compassion
- Assurance that appropriate steps are being taken to prevent the adverse event from happening to another patient in the future

Reporting allows for errors to be studied so that system-based improvements can be made to help prevent such errors from harming patients in the future. Reporting errors is essential for error prevention and provides opportunities to improve processes of care by learning from failures of the health care system. In order to be effective, reporting must be safe. Individuals who report incidents must not be punished or suffer other ill effects from reporting. The fear of punitive retaliation or other negative consequences will serve as an impediment to incident reporting. The identities of reporters should not normally be disclosed to third parties.

Other barriers to error reporting include having the belief that that no corrective action will be taken and having an overly burdensome reporting system. To overcome these barriers, reported events should be reviewed and acted upon in a timely fashion, and the system for reporting errors should be made as straightforward as possible.

One other recognized barrier to error reporting is failure to recognize that an error has occurred. For example, an interventional cardiologist accidentally orders the wrong dose of medication during a cardiac catheterization; however, the nurse who has worked with this cardiologist for years knows the correct dose intended and makes the appropriate adjustment. No harm has occurred but it would be wrong not to realize that an error did happen.

Health care professionals need to be educated about medical error identification, including the identification and importance of near-misses. Although near-misses (errors that occur but fortunately do not result in patient harm) do not generally need to be disclosed to patients, they should still be reported to the system so that they, too, can be studied. One person's near-miss may be the next person's fatal error. Estimates of the scope of medical errors likely do not reflect the numerous near-misses that do not result in patient harm.

It is important to have a culture that promotes error reporting and error analysis in order to enhance health systems. Every error represents an opportunity to improve a process; however, in order to improve, these errors must be recognized and made known so that system-wide learning and performance can take place.

Note

Estimates are that voluntarily reported medical errors only reflect 10–20% of actual errors.

ANALYSIS OF MEDICAL ERRORS

A systematic approach for understanding the cause of adverse events and identifying flaws in the system which can be corrected to prevent harm in the future is called **root cause analysis** (RCA). RCA is *retrospective* in nature; the focus is on systems and process rather than individual blame. The question asked is, "**how did this happen?**" not "whose fault is this?" The goal is to determine why an event happened and what can be done to prevent it from happening again. RCA is not applicable to negligence or willful harm.

A classic tool used in RCA is the **fishbone** or **Ishikawa diagram** (also known as a **cause and effect diagram**), which analyzes a complex system and identifies possible causes for an effect or problem. This type of diagram is used to explore and display all the possible causes of a particular error.

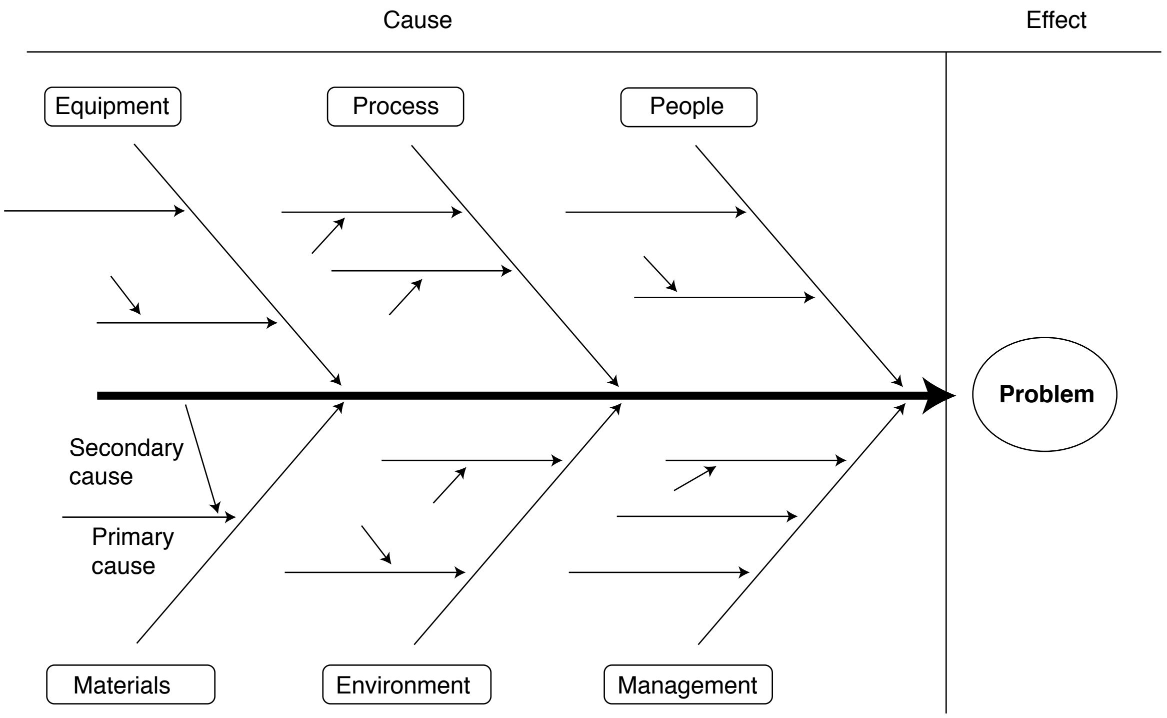


Figure 14-4. Sample Fishbone (Ishikawa) Diagram

The RCA allows the team to identify problems in the system or process of care. The end product of the RCA process is a list of recommended actions to prevent the recurrence of the adverse event in the future. Recommendations commonly consist of one or more of the following actions: standardizing equipment, using double checks or backup systems, employing forcing functions that physically prevent users from making common mistakes, making changes to the physical set-up, updating or improving technology, using cognitive aids (e.g., checklists or mnemonic devices), simplifying a process, educating staff, or implementing new safety policies.

In contrast to the retrospective nature of RCA, the prospective **failure mode effects analysis** (FMEA) is an engineering approach which seeks to anticipate and prevent adverse events through safety design. The goal of FMEA is to prevent patient problems before they occur. FMEA is a systematic and proactive approach that seeks to identify possible failures in the system and potential weaknesses in order to develop strategies to prevent the failures from occurring.

PRINCIPLES OF QUALITY IMPROVEMENT

> "Every system is perfectly designed to get the results it gets."
>
> — Source: Don Berwick, M.D.

A key principle of quality improvement is to design systems capable of identifying, preventing, absorbing, and mitigating errors. Some everyday examples of safety design outside of health care are seatbelt alarms in cars, heat-sensitive fire sprinkler systems, and tip-over switches which automatically turn off space heaters that have accidentally fallen over.

In 2001, the IOM published a report, "Crossing the Quality Chasm," which aimed at promoting fundamental changes in health care in order to close the quality gap. The report recommended a redesign of the American health care system and provided principles for guiding quality improvement. Specifically, the report defined 6 aims of health care (STEEEP):

1. **Safe**: avoidance of injuries to patients from the care that is intended to help them
2. **Timely**: reduce waits and harmful delays in care
3. **Effective**: provide care based on scientific knowledge likely to benefit patients
4. **Efficient**: avoid waste in equipment, supplies, ideas, and energy
5. **Equitable**: provide care that does not vary in quality because of personal characteristics such as gender, ethnicity, geographic location, and socioeconomic status
6. **Patient-centered**: provide care that is respectful of and responsive to individual patient preferences, needs, and values

Another significant initiative in quality improvement is the Institute for Health Care Improvements (IHI) Triple Aim that describes an approach to optimizing health system performance using new designs to pursue 3 dimensions (i.e., "Triple Aim").

- Improve the patient experience of care (including quality and satisfaction)
- Improve the health of populations
- Reduce per capita cost of health care

Measures of Quality

There are three traditional categories of measures used in quality improvement: structure, process, and outcomes.

- **Structure** relates to the physical equipment, resources, or facilities (e.g., number of ICU beds in a hospital).
- **Process** relates to how the system works (e.g., how often nurses use bar coding to identify patients prior to administering medication).
- **Outcomes** represent the final product or end result in patient care (e.g., infection rate in pediatric hematology patients admitted to the hospital). Outcomes are often difficult to assess in quality improvement, and many people often use process measures as a surrogate for outcomes. For example, it may be difficult to accurately track all HAI (outcomes measure), so rates of compliance with hand washing are monitored instead (process measure).

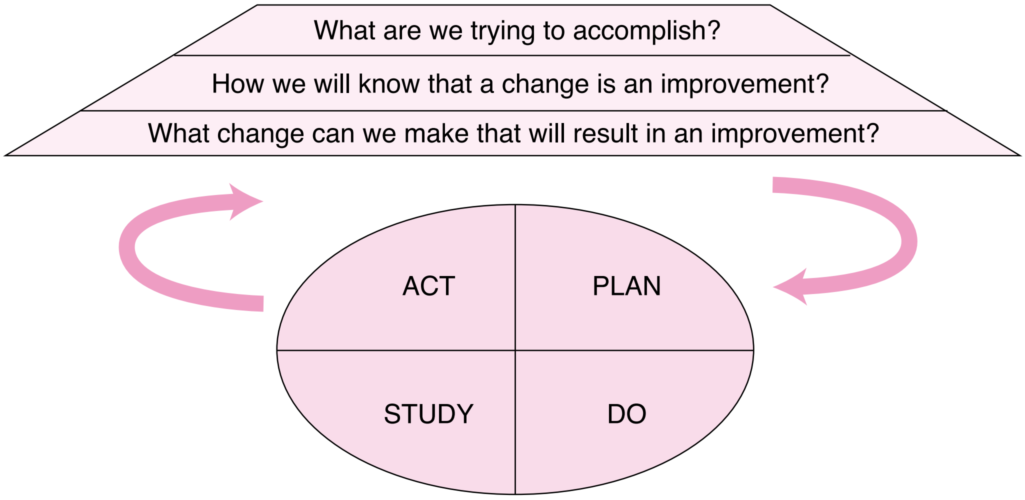
A fourth type of measure introduced to quality improvement is the concept of a balancing measure. **Balancing measures** ask whether changes made to improve one part of the system cause an unanticipated decrease in performance in another part of the system (e.g., did an initiative aimed at increasing the efficiency of discharging patients from the hospital lead to more patients being sent home without appropriate follow-up instructions?).

Models of Quality Improvement

One example of a common quality improvement model is a combination of building and applying knowledge to make an improvement by asking 3 questions and using the **PDSA** (plan, do, study, act) cycle developed by W. Edwards Deming, a pioneer and influential leader in quality control.

1. What are we trying to accomplish?
2. How will we know whether a change is an improvement?
3. What changes can we make that will result in an improvement?

This model takes the simple concept of "trial and error" and transforms it into the PDSA model that can be used to make improvements in health care.



Source: Langley, Nolan, Nolan & Provost 1999

Figure 14-5. The Model for Improvement

- **Plan**: plan a change or a pilot test of a new intervention or innovation
- **Do**: carry out the plan
- **Study**: evaluate the results
- **Act**: decide what actions should be taken to improve (i.e., implement the new intervention or start over with a new plan based on the prior results)

Six Sigma is another model for quality improvement with origins from the manufacturing industry. The term comes from the use in statistics of the Greek Letter (sigma) to denote Standard Deviation from the mean. Six sigma is equivalent to 3.4 defects (or errors) per million. This system uses specific steps to reduce variation and improve performance.

- DMAIC (define – measure – analyze – improve - control): an improvement system for **existing processes** falling below specification
  - **Define**: Define the problem in detail.
  - **Measure**: Measure defects (in terms of "defects per million," or Sigma level).
  - **Analyze**: Do in-depth analysis using process measures, flow charts and defect analysis to determine the conditions under which defects occur.
  - **Improve**: Define and test changes aimed at reducing defects.
  - **Control**: What steps will you take to maintain performance?

Lean (also called Lean Enterprise or Toyota Production System) is an improvement process that seeks to improve value from the patient's perspective, by reducing waste in time and resources that do not enhance patient outcomes. This includes certain lab tests, imaging studies or care services that may be commonly performed, but in reality do not actually help the patient. For example, a pre-operative EKG obtained on a healthy 21 year-old with no cardiac symptoms undergoing a small outpatient procedure can be considered a wasteful test that does not help the patient.

Flowcharts allow health care teams to understand the steps involved in the delivery of patient care service. A flowchart is a visual illustration of all the steps or parts of a process in patient care. There are 2 types of flowcharts: **high-level flowcharts** (more conceptually focused, 'big picture') and **detailed flowcharts** (more focused on specific, fine points).

Flowcharts are more accurate and effective when all representative members of a health care team actively participate in their design. They help health-care providers achieve a shared understanding of a clinical process and use that knowledge as the basis for designing new ways to improve services. Specifically, they can help to identify steps that do not add value to the process (e.g., unnecessary duplication of services), to determine areas of delay in care, and to discover failure points in the system.

Pareto charts are used to describe a large proportion of quality problems being caused by a small number of causes. It is based on the classic 80/20 rule from economics, where 80% of the world's wealth is described to be in the hands of an elite 20% of the population.

The Pareto principle applied to health care states that the **majority of patient safety errors stem from only a few recurring contributing factors**, which should serve as the focus of the problem-solving efforts. In essence, it is a method of prioritizing problems, highlighting the fact that most problems are affected by a few factors and indicating which problems to solve and in what order. A Pareto chart includes the multiple factors that contribute to an effect arranged in descending order (according to the magnitude of their effect). The ordering is an important step because it helps the team concentrate efforts on those factors that have the greatest impact.

Run charts (or time plots) are graphs of data collected over time which can help determine whether an intervention or enhancement in the patient care process has resulted in true improvement over time or rather if it simply represents a random fluctuation (that might be incorrectly interpreted as a significant improvement). Run charts are created by plotting time along the x-axis (e.g., minutes, hours, days, months) and the quality measure on the y-axis (e.g., number of infections, wait times, falls). The median (or 50th percentile) is measured using baseline historical data and then compared to outcomes measured following the quality improvement intervention.

Run charts help identify whether there is a true trend vs. a random pattern. A shift in the process signaling a significant change in quality can be identified, for example, by observing ≥6 consecutive points above or below the median, or by ≥5 consecutive points all increasing or decreasing.

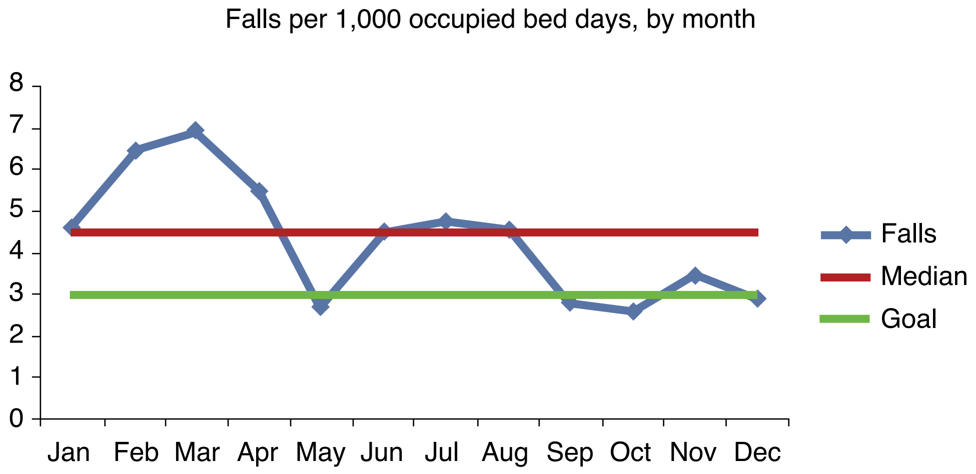


Figure 14-6. Sample Run Chart Plotting Patient Falls (ahrq.gov)

A Shewhart (or control chart) applies formal statistical calculations (statistical process control) to determine whether the observed rise and fall of a quality measure over time is within a predictable range of variation or is an indication of a significant change in the system. Control charts use upper and lower control limits (sometimes called "natural process limits") which indicate the threshold at which the process output is considered statistically 'unlikely,' and are drawn typically at 3 standard errors from the center line.

A **convenience sample** is the study group or population used in the test of a quality improvement initiative. Using convenience sampling is an efficient and simple method to test an intervention. This is not the same process often used in randomized controlled trials, and thus may not be an accurate reflection of the larger group. Ideally, however, the sample will have approximately similar characteristics to the larger population.

LEADING CHANGE IN PATIENT SAFETY

Patient safety is the professional responsibility of everyone on the patient care team. In order to effect change in the quality of health care, health care professionals must utilize leadership principles. Changing behavior is difficult, and there are always multiple barriers to change efforts. To be effective in leading transformation, change efforts need to create a sense of urgency, be data-driven, team-based, specific, and measurable.

Successful leadership can be achieved even without a formal position of authority. Strategies for effectively influencing change include the following:

- Gathering compelling data
- Adopting a 'systems' view of the problem
- Getting buy-in from administrative leadership or a powerful clinical ally
- Developing ideas to solve the problem
- Formulating an action plan

Goals should be SMART (**s**pecific, **m**easurable, **a**chievable, **r**ealistic, and **t**ime-sensitive). Good leaders are able to organize a team, articulate clear goals, manage conflict resolution, and make decisions based on the input of team members. Good leaders also lead by example and model good patient safety behavior.

KEY DEFINITIONS

- **Adverse event:** any injury caused by medical care
  - An adverse event results in unintended harm to a patient by an act of commission or omission, rather than by the underlying disease or condition of the patient. Identifying something as an adverse event does not imply error, negligence, or poor quality care. It simply indicates that an undesirable clinical outcome resulted from some aspect of diagnosis or therapy, not an underlying disease process.
- **Adverse reaction:** occurs when unexpected harm results from a justified action
  - An adverse drug reaction occurs when the correct process was followed for the context in which the medication was used.
- **Authority gradient**: command hierarchy of power or balance of power, measured in terms of steepness
  - First used in aviation to describe the phenomenon where pilots and copilots failed to communicate effectively in stressful situations due to the significant difference in their perceived expertise or authority.

Hierarchies which exist in medicine are also subject to causing errors. Most health care teams require some degree of authority gradient; otherwise roles are blurred and decisions cannot be made in a timely fashion. However, within a hierarchy, tools of effective clinical communication and teamwork can overcome risks to patient safety.

- **Brief**: short planning session prior to the start of a clinical activity, in order to achieve team orientation, establish expectations, anticipate problems, and plan for contingencies
- **Checklist**: algorithmic listing of actions to be performed in a given clinical setting, with the goal to ensure that no critical step will be forgotten
  - Though a seemingly simple intervention, checklists have played a leading role in the most significant successes of the patient safety movement, including the near-elimination of central line–associated bloodstream infections in many intensive care units. Checklists have also been used in the operating room to ensure that OR teams are well-oriented and that evidence-based standards known to reduce complications are followed (e.g., use of pre-operative antibiotics).
- **Closed-loop communication:** a type of communication whereby, when a request is made of team members, someone specifically affirms out loud that he will complete the task and states out loud when the task has been completed
  - For example, during a cardiac resuscitation a physician orders a medication to be given intravenously and the nurse verbally confirms receipt of the order and verbally confirms when the medication has been administered as requested.
- **Debrief:** information exchange process designed to improve team performance and effectiveness, held after a clinical event in order to review and learn
- **Error**: failure of a planned action to be completed as intended or the use of a wrong plan to achieve an aim (i.e., an act of commission or doing something wrong); also includes failure of an unplanned action that should have been completed (i.e., an act of omission, or failing to do the right thing)
  - For example, ordering a medication for a patient with a documented allergy to that medication is an error of commission. Failing to prescribe a proven medication with major benefits for an eligible patient (e.g., low-dose unfractionated heparin for venous thromboembolism prophylaxis for a patient after hip replacement surgery) is an **error of omission**. Errors of omission are more difficult to recognize than errors of commission but are thought to represent a larger scope of the problem in patient safety. Errors can also be defined in terms of active or latent as coined by Professor James Reason, one of the founders in the field of safety science. According to Professor Reason, **active errors** occur at the point of contact between a human and some aspect of a larger system (e.g., a human–machine interface). They are generally readily apparent (e.g., pushing an incorrect button, ignoring a warning light) and almost always involve someone at the frontline. Active errors or active failures are sometimes referred to as

errors at the 'sharp end,' figuratively referring to a scalpel. In other words, errors at the sharp end are noticed first because they are committed by the person closest to the patient. This person may literally be holding a scalpel (e.g., an orthopedist operating on the wrong leg) or figuratively be administering any kind of therapy (e.g., a nurse programming an intravenous pump with the wrong medication dose) or performing any aspect of care.

Latent errors (or latent conditions), in contrast, refer to less apparent failures of organization or design that contribute to the occurrence of errors or allow them to cause harm to patients. To complete the metaphor, latent errors are those at the other end of the scalpel—the 'blunt end'—referring to the many layers of the health care system that affect the person "holding" the scalpel. For example, policies that allow a patient to enter an operating room and start an operation before confirming the patient's identify, intended procedure, and site of surgery are considered latent errors that can result in wrong patient or wrong site surgery.

- **Forcing function**: aspect of a design which prevents a specific action from being performed or allows its performance only if another specific action is performed first
  - For example, automobiles are now designed so that the driver cannot shift into reverse without first putting a foot on the brake pedal. One of the first forcing functions identified in health care was the removal of concentrated potassium from general hospital wards. This action is intended to prevent the inadvertent preparation of intravenous solutions with concentrated potassium, an error that had produced small but consistent numbers of deaths for many years.
- **Handoffs**: the process whereby one health care professional updates another on the status of ≥1 patients for the purpose of taking over their care
  - An example includes a resident physician who has been on call overnight telling an incoming resident about the patients admitted who require ongoing management. Nurses commonly conduct a handover at the end of their shift, updating the oncoming nurse about their status of the patients and tasks that need to be performed. Handoffs are a potential source of patient safety failure from the lack of important information being conveyed or misinformation being conveyed.
- **Harm/hazard**: harm is the impairment or any negative effect on the structure of function of the body (e.g., disease, injury, suffering, disability, death); hazard is a circumstance, agent or action with the potential to cause harm
- **Huddle:** an often impromptu problem-solving meeting conducted in order to assess a critical situation, reestablish situation awareness, reinforce plans already in place, and determine the need to adjust the plan
- **Iatrogenic:** an adverse effect of medical care, rather than of the underlying disease (literally "brought forth by healer," from Greek *iatros*, for healer, and *gennan*, to bring forth)

- **Incident reporting**: collecting and analyzing information about an event which could have harmed (near-miss) or did harm (adverse event) a patient in a health care setting
- **Medication error**: any preventable event which may cause or lead to unintended and incorrect medication use or patient harm, while the medication is in the control of the health care professional or patient
  - Medication error occurs when a patient receives (a) the wrong medication, or (b) the right medication but in the wrong dosage or manner (e.g., given orally instead of IV, correct medication given at the wrong time).
- **Medication reconciliation**: process of avoiding unintended inconsistencies in medication regimens which can occur with any transition in care (e.g., hospital admission, transfer to ICU, discharge to rehab center) by reviewing the patient's current medication regimen and comparing it with the regimen being considered for the new setting of care
  - Medical reconciliation helps to ensure that the intended medications are continued, and that medications that are supposed to be discontinued are not inadvertently continued. For example, medical reconciliation performed prior to discharging a patient from the hospital can detect if medication changes were made during the hospital stay that need to be continued once the patent is home (e.g., intravenous antibiotics started in the hospital during the treatment of pneumonia which are intended to be continued orally at home).
- **Near-miss (or close call)**: error or other incident which does not produce patient injury, but only because of intervening factors or pure chance
  - This good fortune might reflect robustness of the patient (e.g., a patient with penicillin allergy receives penicillin, but has no reaction) or a fortuitous, timely intervention (e.g., a nurse happens to realize that a physician wrote an order in the wrong chart).
- **Patient safety**: The WHO defines patient safety as 'the reduction of risk of unnecessary harm associated with health care to an acceptable minimum (2009)
  - The Agency for Healthcare Research and Quality uses the definition that 'patient safety is a discipline in the health care sector that applies safety science methods toward the goal of achieving a trustworthy system of health care delivery.' Patient safety is also an attribute of health care systems; it minimizes the incidence and impact of, and maximizes recovery from, adverse events.
- **Quality assurance (QA):** an older term, not likely to be used today; QA was reactive, retrospective, policing, and in many ways punitive; often involved determining who was at fault after something went wrong
- **Quality improvement (QI):** involves both prospective and retrospective reviews; is aimed at improvement—measuring where you are and figuring out ways to make things better; specifically attempts to avoid attributing blame and to create systems to prevent errors from happening
- **Read backs (or call-backs)**: when a listener repeats key information so that the transmitter can confirm its correctness

  - To address the possibility of miscommunication when information is conveyed verbally, many high-risk industries use protocols for mandatory read-backs. For example, a laboratory technician calling a physician with a critical lab value may request that the physician read back the critical lab value to ensure it was received correctly.
- **Root cause analysis (RCA):** structured process for identifying the causal or contributing factors underlying adverse events or other critical incidents
  - Initially developed to analyze industrial accidents, RCA is now widely employed in error analysis within health care. A central tenet of RCA is to identify underlying problems that increase the likelihood of errors, while avoiding the trap of focusing on mistakes by individuals. RCA seeks to explore all the possible factors associated with an incident by asking what happened, why it happened, and what can be done to prevent it from happening again.
- **SBAR:** a form of structured communication first developed for use in naval military procedures; it stands for situation (what is going on with the patient?), background (what is the clinical background or context?), assessment (what do I think the problem is?), recommendation/request (what would I do to correct it?)
  - It has been adapted for health care as a helpful technique for communicating critical information that requires immediate attention and action concerning a patient's condition. It promotes patient safety by helping individuals communicate with shared expectations in a concise and structured format which improves efficiency and accuracy.
- **Sentinel event:** adverse event in which death or serious harm to a patient has occurred; used to refer primarily to events that were not at all expected or acceptable (e.g., an operation on the wrong patient or body part)
- **Violation:** intentional or deliberate deviation from safe operating procedures, standards, or policies
  - A violation is different from an error, which is an unintentional action. Unlike errors which are honest mistakes due to human nature, intentional violations are behaviors for which individuals need to be held accountable.
- **Wrong-site procedure**: operation or procedure done on the wrong part of the body or on the wrong person; it can also mean the wrong surgery or procedure was performed
  - Wrong-site procedures are rare and preventable, though they do still occur. A standard system to confirm the patient, site, and intended procedure with the medical team and patient before starting the procedure is a widely employed method of reducing or eliminating wrong-site procedures.

Review Questions

1. A 64-year-old man is admitted to the hospital for treatment of bacterial pneumonia. The treating clinician forgets to ask about allergies and the patient is unaware that his severe allergy to penicillin is not known to the treatment team. The patient receives a dose of intravenous penicillin and suffers an anaphylactic response but is successfully resuscitated by the medical team. Which of the following is the most accurate description of the medical error?

   A. Slip resulting in a near miss
   B. Violation resulting in a near miss
   C. Lapse resulting in an adverse event
   D. Violation resulting in patient injury
   E. Non-preventable adverse event

2. A new intern who is not being supervised is asked to see a patient who is being discharged following treatment of a lower extremity deep vein thrombosis. The intern prescribes a 6-month course of warfarin without reviewing the patient's other medications. Unknown to the intern, the patient is taking an antibiotic which increases the anticoagulant activity of warfarin. The pharmacy computer system is broken and the drug is filled manually, which does not enable the computer system to alert to the drug–drug interaction. An overworked nurse fails to check for drug interactions during the medication reconciliation and gives the patient the prescription upon discharge. The patient fills the prescription at a local drug store and suffers a significant bleeding complication requiring re-hospitalization and surgery.

   According to James Reason's Swiss cheese model of error, which one of the following would be the most effective approach to preventing this adverse event in the future?

   A. Identifying and correcting the single overarching failure in the health care system responsible for the adverse event
   B. Identifying and weeding out the individuals involved in this medical error
   C. Applying a systemic approach to eliminating all causes of human error
   D. Building successive layers of safety barriers into the system that prevent medical error from resulting in patient harm
   E. Initiating a campaign to remind clinicians to be more vigilant and to follow established safety protocols

3. As a member of the clinical team you are interested in developing a new system to more closely monitor blood glucose levels of diabetic patients admitted to the hospital for vascular wound care. In order to assess the impact of this new system on patient quality, you and your team conduct a PDSA cycle. Which of the following statements is correct regarding the PDSA cycle?

   A. The PDSA cycle begins with full scale implementation.
   B. The PDSA cycle consists of small, rapid tests of new initiatives.
   C. Changes from PDSA are based on expert intuition, and do not require data collection or interpretation.
   D. PDSA is a means of analyzing past errors in order to design system based interventions.
   E. The PDSA cycle requires a randomized control trial.

4. A 23-year-old man with a history of depression is admitted to the inpatient psychiatry ward after the third attempt at suicide with an intentional drug overdose. The patient has been stabilized medically; however, is under 24-hour monitoring by the nursing staff due to repeated attempts at self-harm. During change of shift, there is a mistake in communication and no one is assigned to the patient. The mistake is noticed 15 minutes into the new shift, and a member of the nursing team is assigned to watch the patient. Fortunately, during the 15-minute period the patient did not make any attempts to harm himself. Which of the following statements about this event is correct?

   A. This is a sentinel event and should be reported to the medical board.
   B. This is a sentinel event and should be reported to the hospital and family.
   C. This is a near miss and should be reported to the hospital.
   D. This is a near miss and should be reported to the patient and family.
   E. This is a near miss and no reporting is required since the patient was not harmed.

5. A nurse practitioner receives a phone call from the mother of one of the pediatric patients in the practice who frequently suffers from ear infections. The mother typically sees the physician and receives antibiotics to treat her child's condition. Given that it is a weekend and the office is closed, the nurse practitioner phones in the antibiotic prescription based on the mother's recollection of the name of the medication used in the past. The prescription is filled at a new pharmacy that does not have the patient's prior medical records on file. The child suffers an allergic reaction after which it is discovered that the wrong antibiotic was ordered. Which of the following statements is correct regarding root cause analysis (RCA)?

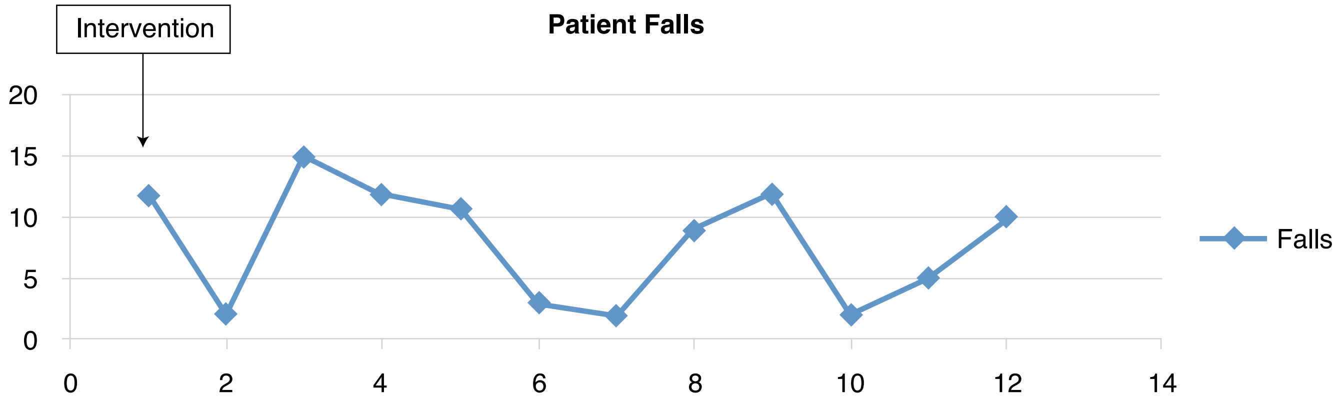
   A. RCA involves a retrospective, systems approach to error analysis.
   B. RCA is a prospective approach to systems redesign.
   C. RCA involves only the individual(s) directly involved in the error.
   D. RCA is only performed for errors resulting in patient death.
   E. Due to privacy laws the results of RCA are confidential and not shared.

6. A critical and respiratory care unit is attempting to decrease their rate of ventilator-acquired pneumonia. The team develops a new clinical protocol to help reduce hospital-acquired pneumonia in ventilated patients. The protocol includes several new activities which have not previously been followed uniformly in the unit. The changes includes head of bed elevation, daily oral care, daily assessment of readiness to extubate and having access to infectious disease specialists for consultation in the treatment of ventilator-associated pneumonia.

   Which one of the following represents an outcomes measure of quality?

   A. Measure the compliance rate in following guidelines for head of bed elevation over a three-month period following the new protocol.
   B. Determine the number of infectious disease specialists available for consultation during a three-month period following the new protocol.
   C. Monitor the number of patients who self-extubate prematurely during the daily assessment of readiness to extubate over a three-month period following implementation of the new protocol.
   D. Monitor the number of infections over three months following implementation of the new protocol.
   E. Determine the wait time for starting antibiotics in patients with suspected ventilator-associated pneumonia.

7. A geriatric team is interested in decreasing the number of patient falls in their nursing home. After convening as a group to discuss possible interventions, a new system of identifying patients at high risk for falling and providing these patients with fall prevention interventions is implemented. Following this intervention, the rate of patient falls per month is followed for 12 months on a run chart. No baseline data was collected. Which of the following best describes the results of the run chart?



   A. The intervention led to a significant decrease in patient falls.
   B. The intervention led to a significant increase in patient falls.
   C. The intervention resulted in no change in patient falls.
   D. The impact of the intervention is subjective.
   E. The impact of the intervention is inconclusive.

8. An 85-year-old woman is being transferred to an acute rehabilitation facility following a hospital admission for hip replacement surgery. Postoperatively during her hospital stay she was started on deep vein thrombosis (DVT) prophylaxis medication with plans to continue the medication upon discharge. The intern and nurse discharging the patient failed to convey this new medication to the receiving treatment team at the rehabilitation center. The patient is not continued on her anticoagulation medication and sustains a DVT leading to a fatal pulmonary embolus 3 weeks after transfer. Which of the following actions will facilitate quality improvement and the prevention of a similar error in the future?

   A. Determine which staff member(s) failed to order the medication.
   B. Develop a process to increase the use of medication reconciliation.
   C. Send a memo to all staff about the importance of DVT prophylaxis.
   D. Educate patients about the dangers of DVT following hip surgery.
   E. Conduct monthly audits to monitor medication errors at transitions of care.

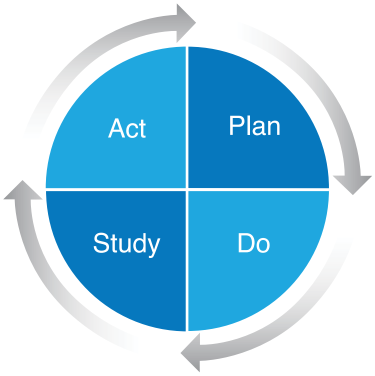
Answers and Explanations

1. **Answer: C.** A lapse is an internal event that generally involves a failure in memory; as opposed to a slip which is an observable action commonly associated with attentional or perceptional failures resulting in an unintended execution of a correctly intended action. The result of the described error was harm to the patient in the form of anaphylaxis with the need for resuscitation, and therefore is categorized as an adverse event. An adverse event is any harm or undesirable clinical outcome resulting from medical care as opposed to the underlying disease process, and does not have to result in permanent disability or death. A violation is a deliberate act of not following policy or procedures, which was not the situation in this scenario. A near miss is an event or a situation that does not produce patient harm, but only because of intervening factors or good fortune. The adverse event described is the result of an error and is completely preventable.

2. **Answer: D.** The most effective approach to improving patient safety and quality is to address system-level causes of failure. In the Swiss cheese model, safety barriers are recognized as having unintended weaknesses (i.e., holes) which can occasionally align and allow an error to result in patient harm. In order to improve patient safety, the system must be redesigned to have effective safety barriers capable of preventing errors from resulting in patient harm. Attempting to penalize individuals who make honest errors or eliminate the potential for human error yields limited results.

3. **Answer: B.** The PDSA Cycle is a systematic series of steps for gaining valuable learning and knowledge for the continual improvement of a product or process The PDSA cycle consists of developing a plan to test an intervention (Plan), carrying out the intervention (Do), observing and measuring the impact of the intervention (Study), and determining what modifications should be made to the system or process as a result of the study observations (Act). These interventions are small scale, rapid tests of new initiatives. Interventions with promising results are then selected for larger scale implementation. They do not require the rigor of

randomized controlled trials. These 4 steps are repeated over and over as part of a never-ending cycle of continual improvement.



4. **Answer: C.** The event described is a near miss; there was an error which fortunately did not result in patient harm. Most near misses need not be disclosed to patients or families; however, they should be reported to the hospital in order for the error to be studied in an attempt to learn how to prevent it in the future. A sentinel event is an adverse event resulting in serious or permanent injury to a patient.

5. **Answer: A.** The root cause analysis is a retrospective approach to error analysis. It is typically performed for errors resulting in significant patient harm, but can be performed for any adverse event that a team wishes to review. The RCA process usually involves the individual(s) involved in the event as well as any other members of the team typically involved in the care delivery process related to the event. Although the details such as the names of the individuals involved in the event are not shared publicly, the general findings of the RCA can be shared throughout the system in order to improve the quality of the system.

6. **Answer: D.** The number of infections over 3 months following implementation of the new protocol is an outcomes measure. Compliance rates in following guidelines are a process measure. The number of infectious disease specialists would be a structure measure. The number of patients prematurely self-extubating would be a balancing measure. Wait times for starting antibiotics is another process measure.

7. **Answer: E.** A run chart provides a dynamic display of a process over time. Run charts help to determine using minimal mathematical complexity if interventions made in a process or system over time lead to improvements. Run charts also provide the foundation for the more sophisticated method of statistical analysis using control charts. The run chart allows a team to understand the stability of a process as well as determine any shifts, trends or runs which may indicate changes based on interventions. However, without a baseline for comparison, one cannot determine from this run chart whether or not any significant change has occurred.

8. **Answer: B.** Quality assurance (QA) is an older term describing a process that is reactive and retrospective in nature. It is a form of 'policing' to ensure that quality standards have been followed. It often relies on audits

and traditionally has focused on punitive actions for failures in quality. It often involves determining who was at fault after something went wrong. QA has not proven to be very effective in transforming care. The goal of quality improvement (QI), on the other hand, is to achieve improvement by measuring the current status of care and then developing systems-based approaches to making things better. It involves both prospective and retrospective reviews and specifically attempts to avoid attributing blame. Rather, QI seeks to create systems to prevent errors from happening. In the case above, developing a process to increase the use of medication reconciliation would be following the principles of QI. The other interventions are QA-based and/or simply not as effective in creating and sustaining a positive change.

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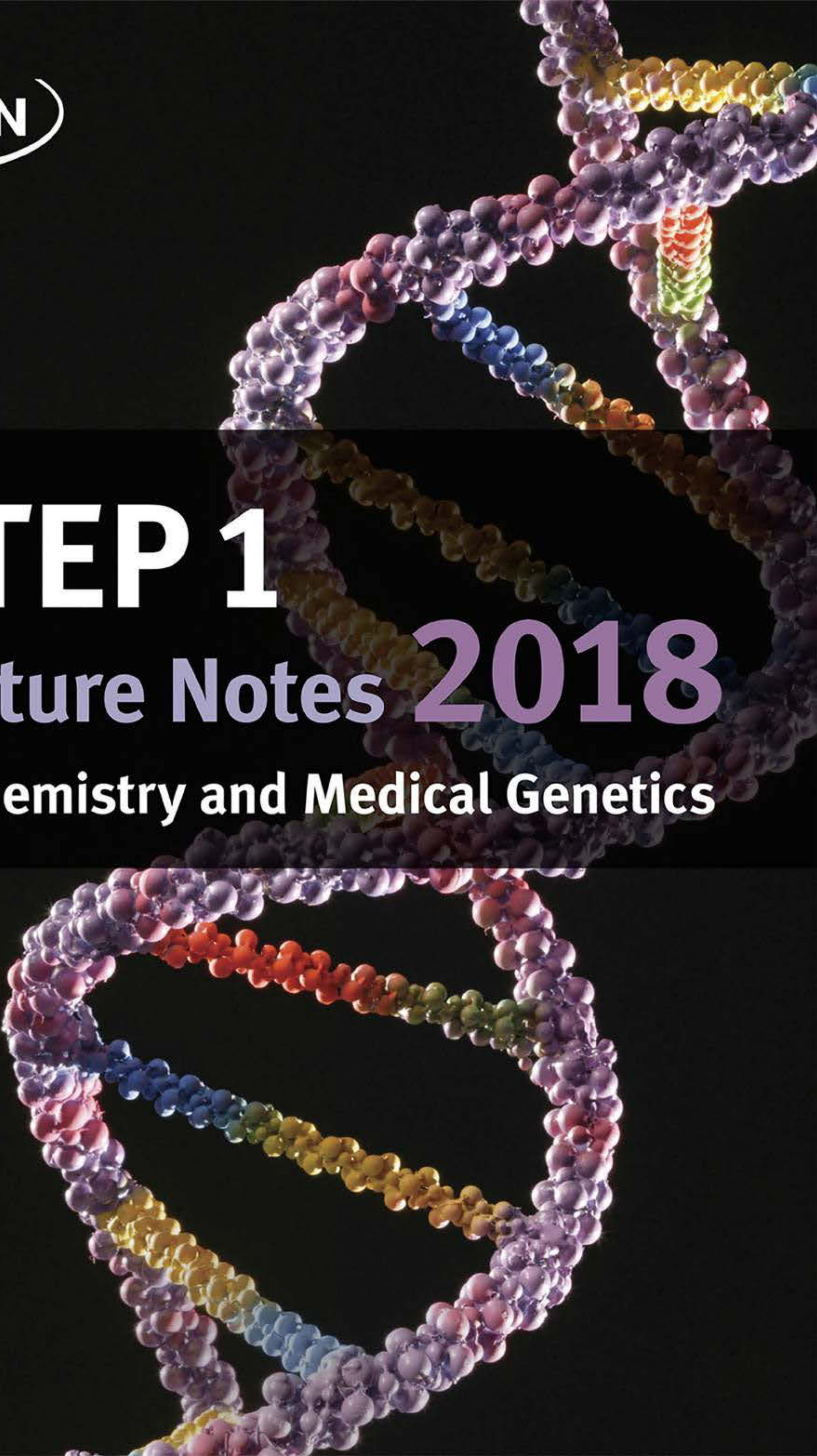

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PART I

Biochemistry

Nucleic Acid Structure and Organization

1

Learning Objectives

- ❑ Explain information related to nucleotide structure and nomenclature
- ❑ Use knowledge of organization of DNA versus RNA
- ❑ Understand general features of a chromosome

CENTRAL DOGMA OF MOLECULAR BIOLOGY

An organism must be able to store and preserve its genetic information, pass that information along to future generations, and express that information as it carries out all the processes of life. The major steps involved in handling genetic information are illustrated by the central dogma of molecular biology.

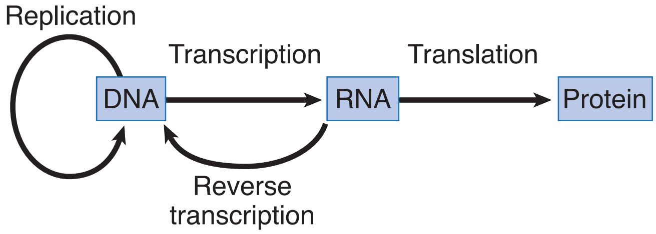


Figure I-1-1. Central Dogma of Molecular Biology

Genetic information is stored in the base sequence of DNA molecules. Ultimately, during the process of gene expression, this information is used to synthesize all the proteins made by an organism.

Classically, a **gene** is a unit of the DNA that encodes a particular protein or RNA molecule. Although this definition is now complicated by our increased appreciation of the ways in which genes may be expressed, it is still useful as a working definition.

Gene Expression and DNA Replication

High-Yield

Gene expression and DNA replication are compared below. Transcription, the first stage in gene expression, involves transfer of information found in a double-stranded DNA molecule to the base sequence of a single-stranded RNA molecule. If the RNA molecule is a messenger RNA, then the process known as translation converts the information in the RNA base sequence to the amino acid sequence of a protein.

When cells divide, each daughter cell must receive an accurate copy of the genetic information. DNA replication is the process in which each chromosome is duplicated before cell division.

Table I-1-1. Comparison of Gene Expression and DNA Replication

Gene Expression	DNA Replication
Produces all the proteins an organism requires	Duplicates the chromosomes before cell division
Transcription of DNA: RNA copy of a small section of a chromosome (average size of human gene, 10^4–10^5 nucleotide pairs)	DNA copy of entire chromosome (average size of human chromosome, 10^8 nucleotide pairs)
Transcription occurs in the nucleus throughout interphase	Occurs during S-phase
Translation of RNA (protein synthesis) occurs in the cytoplasm throughout the cell cycle	Replication in nucleus

The concept of the cell cycle can be used to describe the timing of some of these events in a eukaryotic cell. The M phase (mitosis) is the time in which the cell divides to form 2 daughter cells. Interphase describes the time between 2 cell divisions or mitoses. Gene expression occurs throughout all stages of interphase. Interphase is subdivided as follows:

- G_1 phase (gap 1) is a period of cellular growth preceding DNA synthesis. Cells that have stopped cycling, such as muscle and nerve cells, are said to be in a special state called G_0.
- S phase (DNA synthesis) is the period of time during which DNA replication occurs. At the end of S phase, each chromosome has doubled its DNA content and is composed of 2 identical sister chromatids linked at the centromere.
- G_2 phase (gap 2) is a period of cellular growth after DNA synthesis but preceding mitosis. Replicated DNA is checked for any errors before cell division.

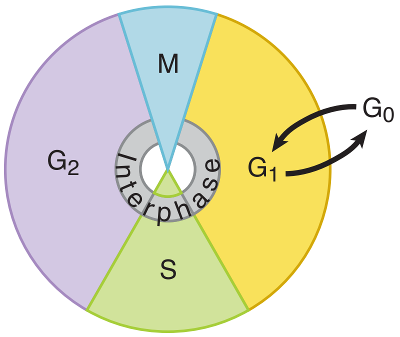


Figure I-1-2. Eukaryotic Cell Cycle

Note

Many chemotherapeutic agents function by targeting specific phases of the cell cycle. This is a frequently tested area on the exam.

Some commonly tested agents with phase of cell cycle they target:

- S-phase: methotrexate, 5-fluorouracil, hydroxyurea
- G2 phase: bleomycin
- M phase: paclitaxel, vincristine, vinblastine
- Non cell-cycle specific: cyclophosphamide, cisplatin

Control of the cell cycle is accomplished at checkpoints between the various phases by strategic proteins such as cyclins and cyclin-dependent kinases. These checkpoints ensure that cells will not enter the next phase of the cycle until the molecular events in the previous cell cycle phase are concluded.

Reverse transcription, which produces DNA copies of an RNA, is more commonly associated with life cycles of retroviruses, which replicate and express their genome through a DNA intermediate (an integrated provirus). Reverse transcription also occurs to a limited extent in human cells, where it plays a role in amplifying certain highly repetitive sequences in the DNA (Chapter 7).

NUCLEOTIDE STRUCTURE AND NOMENCLATURE

Nucleic acids (DNA and RNA) are assembled from nucleotides, which consist of 3 components: a nitrogenous base, a 5-carbon sugar (pentose), and phosphate.

Five-Carbon Sugars

Nucleic acids (as well as nucleosides and nucleotides) are classified according to the pentose they contain. If the pentose is ribose, the nucleic acid is RNA (ribonucleic acid); if the pentose is deoxyribose, the nucleic acid is DNA (deoxyribonucleic acid).

Bases

High-Yield

There are 2 types of nitrogen-containing bases commonly found in nucleotides: purines and pyrimidines.

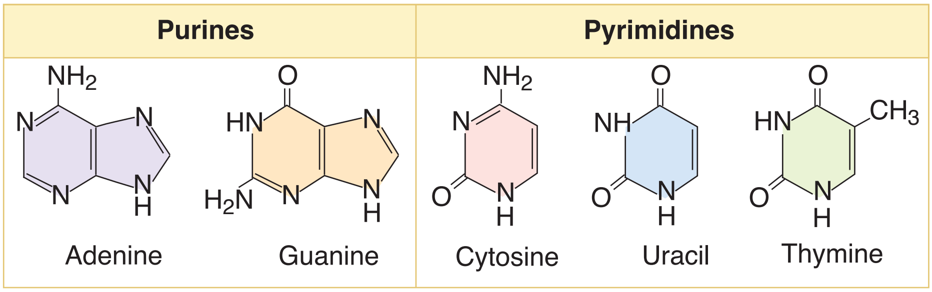


Figure I-1-3. Bases Commonly Found in Nucleic Acids

- Purines contain 2 rings in their structure. The purines commonly found in nucleic acids are adenine (A) and guanine (G); both are found in DNA and RNA. Other purine metabolites, not usually found in nucleic acids, include xanthine, hypoxanthine, and uric acid.
- Pyrimidines have only 1 ring. Cytosine (C) is present in both DNA and RNA. Thymine (T) is usually found only in DNA, whereas uracil (U) is found only in RNA.

Nucleosides and Nucleotides

Nucleosides are formed by covalently linking a base to the number 1 carbon of a sugar. The numbers identifying the carbons of the sugar are labeled with "primes" in nucleosides and nucleotides to distinguish them from the carbons of the purine or pyrimidine base.

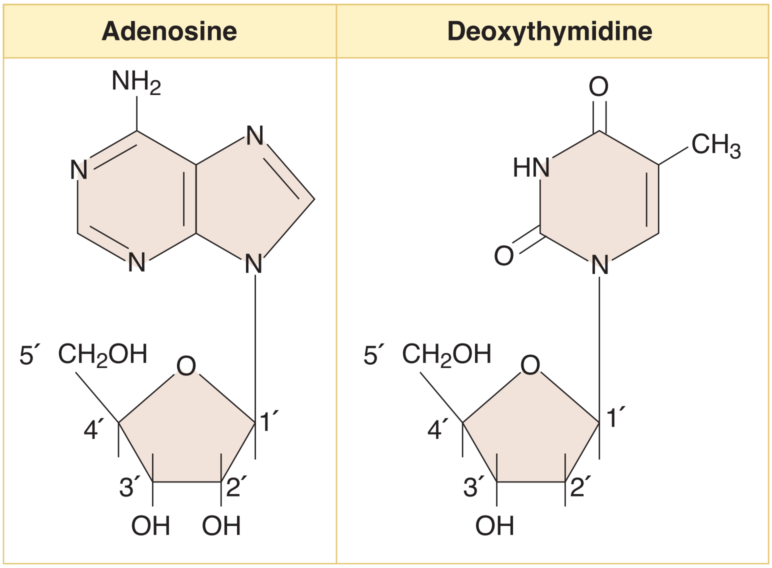


Figure I-1-4. Examples of Nucleosides

Nucleotides are formed when 1 or more phosphate groups is attached to the 5′ carbon of a nucleoside. Nucleoside di- and triphosphates are high-energy compounds because of the hydrolytic energy associated with the acid anhydride bonds.

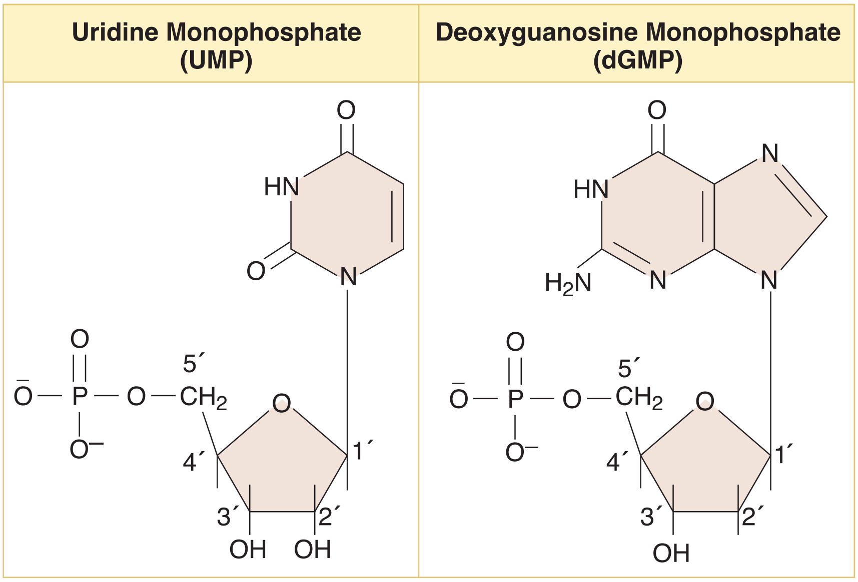


Figure I-1-5. Examples of Nucleotides

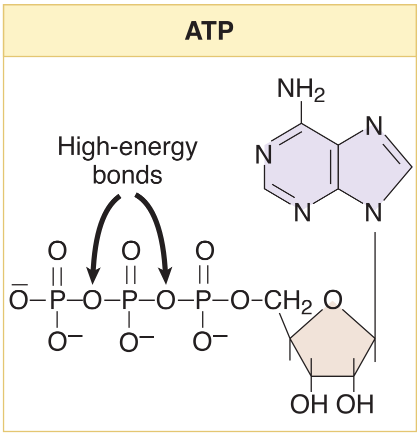


Figure I-1-6. High-Energy Bonds in a Nucleoside Triphosphate

The nomenclature for the commonly found bases, nucleosides, and nucleotides is shown below. Note that the "deoxy" part of the names deoxythymidine, dTMP, etc., is sometimes understood and not expressly stated because thymine is almost always found attached to deoxyribose.

Table I-1-2. Nomenclature of Important Bases, Nucleosides, and Nucleotides

Base	Nucleoside	Nucleotides		
Adenine	Adenosine (Deoxyadenosine)	AMP (dAMP)	ADP (dADP)	ATP (dATP)
Guanine	Guanosine (Deoxyguanosine)	GMP (dGMP)	GDP (dGDP)	GTP (dGTP)
Cytosine	Cytidine (Deoxycytidine)	CMP (dCMP)	CDP (dCDP)	CTP (dCTP)
Uracil	Uridine (Deoxyuridine)	UMP (dUMP)	UDP (dUDP)	UTP (dUTP)
Thymine	(Deoxythymidine)	(dTMP)	(dTDP)	(dTTP)

Names of nucleosides and nucleotides attached to deoxyribose are shown in parentheses.

NUCLEIC ACIDS

Nucleic acids are polymers of nucleotides joined by 3′, 5′-phosphodiester bonds; that is, a phosphate group links the 3′ carbon of a sugar to the 5′ carbon of the next sugar in the chain. Each strand has a distinct 5′ end and 3′ end, and thus has polarity. A phosphate group is often found at the 5′ end, and a hydroxyl group is often found at the 3′ end.

The base sequence of a nucleic acid strand is written by convention, in the 5′→3′ direction (left to right). According to this convention, the sequence of the strand on the left in Figure I-1-7 must be written **5′-TCAG-3′ or TCAG:**

- If written backward, the ends must be labeled: 3′-GACT-5′
- The positions of phosphates may be shown: pTpCpApG
- In DNA, a "d" (deoxy) may be included: dTdCdAdG

In eukaryotes, DNA is generally double-stranded (dsDNA) and RNA is generally single-stranded (ssRNA). Exceptions occur in certain viruses, some of which have ssDNA genomes and some of which have dsRNA genomes.

Note

Nucleic Acids

- Nucleotides linked by 3′, 5′ phosphodiester bonds
- Have distinct 3′ and 5′ ends, thus polarity
- Sequence always specified as 5′→3′

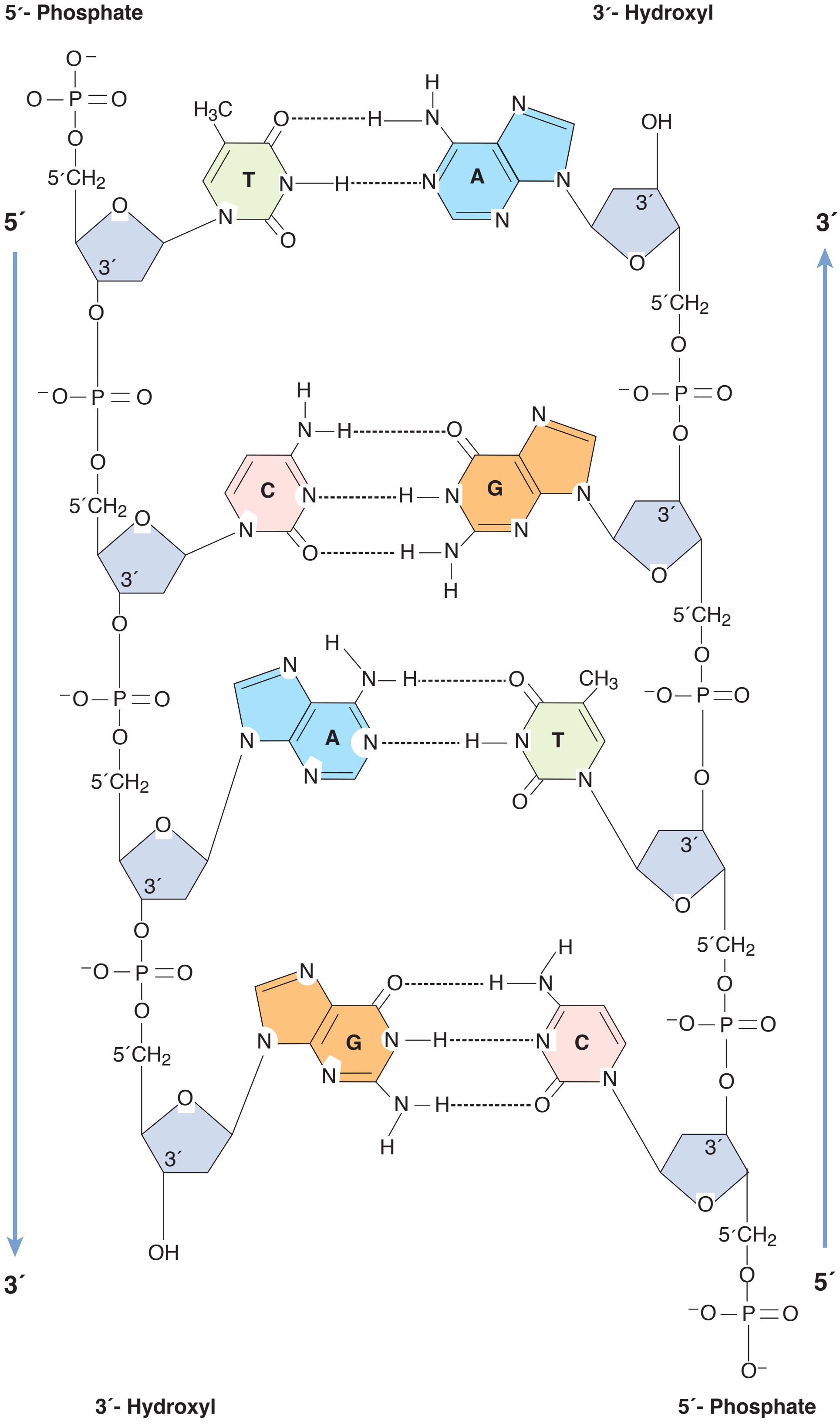


Figure I-1-7. Hydrogen-Bonded Base Pairs in DNA

DNA Structure

Some of the features of double-stranded DNA include:

- The 2 strands are antiparallel (opposite in direction).
- The 2 strands are complementary. A always pairs with T (2 hydrogen bonds), and G always pairs with C (3 hydrogen bonds). Thus, the base sequence on one strand defines the base sequence on the other strand.
- Because of the specific base pairing, the amount of A equals the amount of T, and the amount of G equals the amount of C. Thus, total purines equals total pyrimidines. These properties are known as Chargaff's rules.

With minor modification (substitution of U for T) these rules also apply to dsRNA.

Most DNA occurs in nature as a right-handed double-helical molecule known as Watson-Crick DNA or B-DNA. The hydrophilic sugar-phosphate backbone of each strand is on the outside of the double helix. The hydrogen-bonded base pairs are stacked in the center of the molecule. There are about 10 base pairs per complete turn of the helix. A rare left-handed double-helical form of DNA that occurs in G-C–rich sequences is known as Z-DNA. The biologic function of Z-DNA is unknown, but may be related to gene regulation.

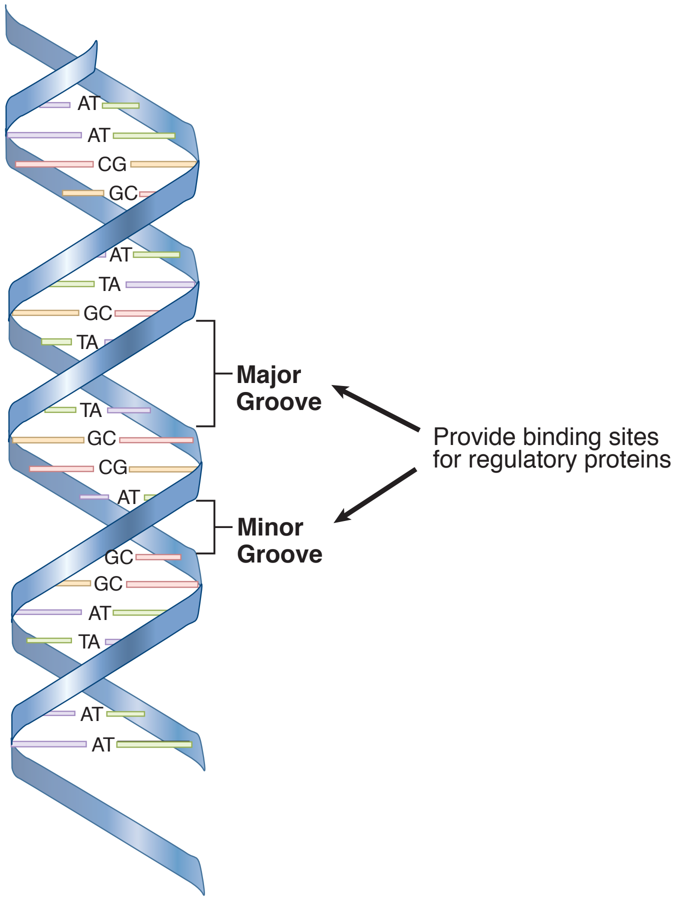


Figure I-1-8. B-DNA Double Helix

Note

Using Chargaff's Rules

In dsDNA (or dsRNA)
(ds = double-stranded)

% A = % T (% U)

% G = % C

% purines = % pyrimidines

A sample of DNA has 10% G; what is the % T?

10% G + 10% C = 20%

therefore, % A + % T must total 80%

40% A and 40% T

Ans: 40% T

Bridge to Pharmacology

Daunorubicin and doxorubicin are antitumor drugs that are used in the treatment of leukemias. They exert their effects by intercalating between the bases of DNA, thereby interfering with the activity of topoisomerase II and preventing proper replication of the DNA.

Other drugs, such as cisplatin, which is used in the treatment of bladder and lung tumors, bind tightly to the DNA, causing structural distortion and malfunction.

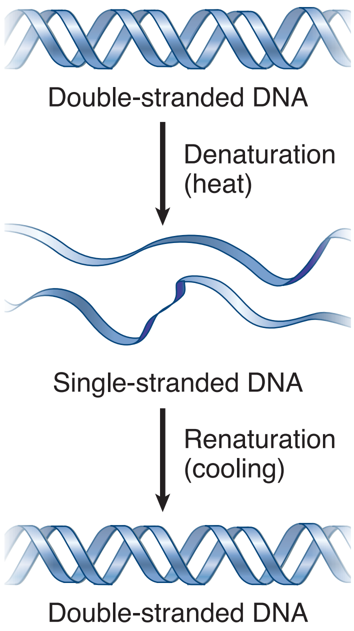


Figure I-1-9. Denaturation and Renaturation of DNA

Denaturation and Renaturation of DNA

Double-helical DNA can be denatured by conditions that disrupt hydrogen bonding and base stacking, resulting in the "melting" of the double helix into two single strands that separate from each other. No covalent bonds are broken in this process. Heat, alkaline pH, and chemicals such as formamide and urea are commonly used to denature DNA.

Denatured single-stranded DNA can be renatured (annealed) if the denaturing condition is slowly removed. For example, if a solution containing heat-denatured DNA is slowly cooled, the two complementary strands can become base-paired again (Figure I-1-9).

Such renaturation or annealing of complementary DNA strands is an important step in probing a Southern blot and in performing the polymerase chain reaction (reviewed in Chapter 7). In these techniques, a well-characterized probe DNA is added to a mixture of target DNA molecules. The mixed sample is denatured and then renatured. When probe DNA binds to target DNA sequences of sufficient complementarity, the process is called hybridization.

Recall Question

Methotrexate affects which portion of the cell cycle?

A. G1 phase

B. G2 phase

C. M phase

D. S phase

Answer: D

ORGANIZATION OF DNA

Large DNA molecules must be packaged in such a way that they can fit inside the cell and still be functional.

Supercoiling

Mitochondrial DNA and the DNA of most prokaryotes are closed circular structures. These molecules may exist as relaxed circles or as supercoiled structures in which the helix is twisted around itself in 3-dimensional space. Supercoiling results from strain on the molecule caused by under- or overwinding the double helix:

- Negatively supercoiled DNA is formed if the DNA is wound more loosely than in Watson-Crick DNA. This form is required for most biologic reactions.
- Positively supercoiled DNA is formed if the DNA is wound more tightly than in Watson-Crick DNA.

- Topoisomerases are enzymes that can change the amount of supercoiling in DNA molecules. They make transient breaks in DNA strands by alternately breaking and resealing the sugar-phosphate backbone. For example, in *Escherichia coli*, DNA gyrase (DNA topoisomerase II) can introduce negative supercoiling into DNA.

Nucleosomes and Chromatin

High-Yield

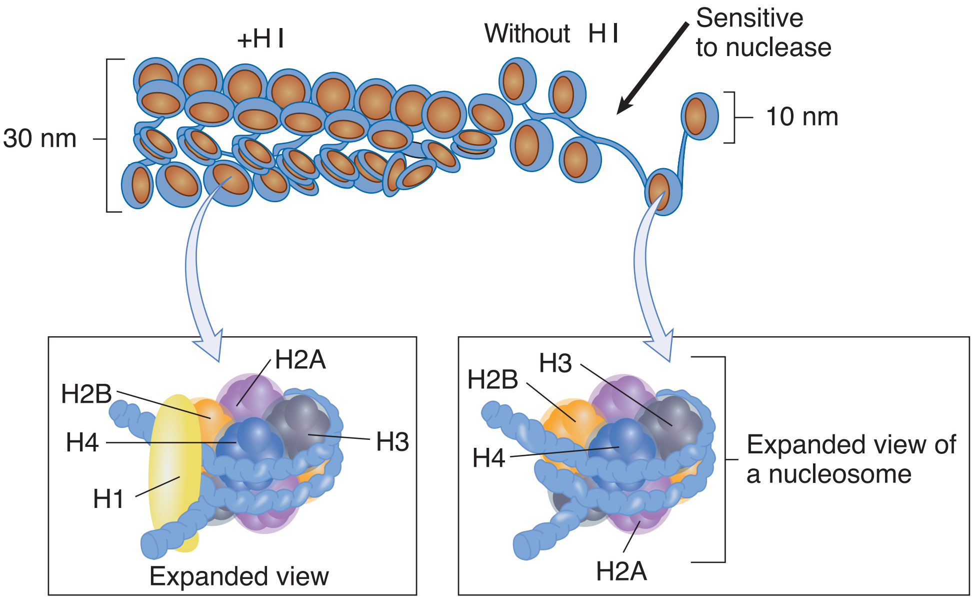


Figure I-1-10. Nucleosome and Nucleofilament Structure in Eukaryotic DNA

Nuclear DNA in eukaryotes is found in chromatin associated with histones and nonhistone proteins. The basic packaging unit of chromatin is the nucleosome.

- Histones are rich in lysine and arginine, which confer a positive charge on the proteins.
- Two copies each of histones H2A, H2B, H3, and H4 aggregate to form the histone octamer.
- DNA is wound around the outside of this octamer to form a nucleosome (a series of nucleosomes is sometimes called "beads on a string" but is more properly referred to as a 10nm chromatin fiber).
- Histone H1 is associated with the linker DNA found between nucleosomes to help package them into a solenoid-like structure, which is a thick 30-nm fiber.
- Further condensation occurs to eventually form the chromosome. Each eukaryotic chromosome in G0 or G1 contains one linear molecule of double-stranded DNA.

Cells in interphase contain 2 types of chromatin: euchromatin (more opened and available for gene expression) and heterochromatin (much more highly condensed and associated with areas of the chromosomes that are not expressed).

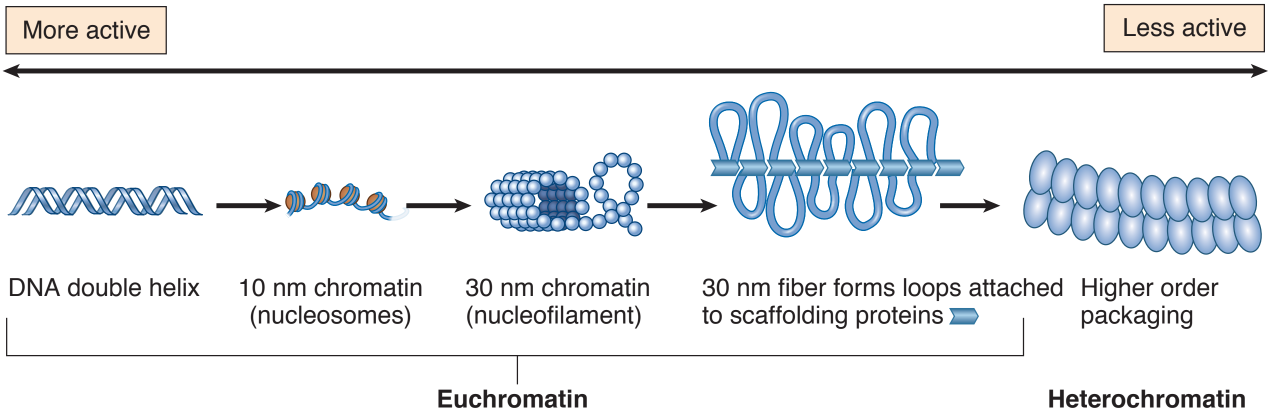


Figure I-1-11. DNA Packaging in Eukaryotic Cell

Euchromatin generally corresponds to the nucleosomes (10-nm fibers) loosely associated with each other (looped 30-nm fibers). Heterochromatin is more highly condensed, producing interphase heterochromatin as well as chromatin characteristic of mitotic chromosomes. The figure below shows an electron micrograph of an interphase nucleus containing euchromatin, heterochromatin, and a nucleolus. The nucleolus is a nuclear region specialized for ribosome assembly (discussed in Chapter 3).

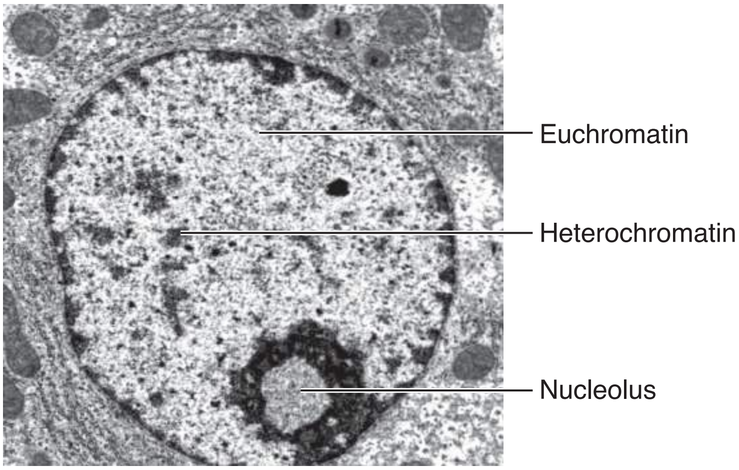


Figure I-1-12. An Interphase Nucleus

During mitosis, all the DNA is highly condensed to allow separation of the sister chromatids. This is the only time in the cell cycle when the chromosome structure is visible. Chromosome abnormalities may be assessed on mitotic chromosomes by karyotype analysis (metaphase chromosomes) and by banding techniques (prophase or prometaphase), which identify aneuploidy, translocations, deletions, inversions, and duplications.

Review Questions

Select the ONE best answer.

1. A double-stranded RNA genome isolated from a virus in the stool of a child with gastroenteritis was found to contain 15% uracil. What is the percentage of guanine in this genome?

   A. 15

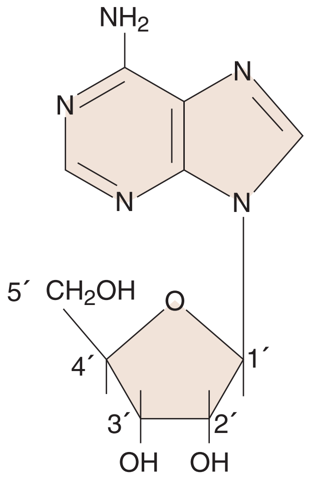
   B. 25

   C. 35

   D. 75

   E. 85

2. What is the structure indicated below?



   A. Purine nucleotide

   B. Purine

   C. Pyrimidine nucleoside

   D. Purine nucleoside

   E. Deoxyadenosine

3. Endonuclease activation and chromatin fragmentation are characteristic features of eukaryotic cell death by apoptosis. Which of the following chromosome structures would most likely be degraded first in an apoptotic cell?

   A. Barr body

   B. 10-nm fiber

   C. 30-nm fiber

   D. Centromere

   E. Heterochromatin

4. A medical student working in a molecular biology laboratory is asked by her mentor to determine the base composition of an unlabeled nucleic acid sample left behind by a former research technologist. The results of her analysis show 10% adenine, 40% cytosine, 30% thymine and 20% guanine. What is the most likely source of the nucleic acid in this sample?

   A. Bacterial chromosome

   B. Bacterial plasmid

   C. Mitochondrial chromosome

   D. Nuclear chromosome

   E. Viral genome

Answers

1. **Answer: C.**

   U = A = 15%.

   Since A + G = 50%, G = 35%.

   Alternatively, U = A = 15%, then U + A = 30%

   C + G = 70%, and

   G = 35%.

2. **Answer: D.** A nucleoside consists of a base and a sugar. The figure shows the nucleoside adenosine, which is the base adenine attached to ribose.

3. **Answer: B.** The more “opened” the DNA, the more sensitive it is to enzyme attack. The 10-nm fiber, without the H1, is the most open structure listed. The endonuclease would attack the region of unprotected DNA between the nucleosomes.

4. **Answer: E.** A base compositional analysis that deviates from Chargaff’s rules (%A = %T, %C = %G) is indicative of single-stranded, not double-stranded, nucleic acid molecule. All options listed except E are examples of circular **(choices A, B and C)** or linear **(choice D)** DNA double helices. Only a few viruses (e.g. parvovirus) have single-stranded DNA.

DNA Replication and Repair

2

Learning Objectives

- ❑ Explain how DNA and RNA synthesis differ
- ❑ Know key steps in DNA replication
- ❑ Know major kinds of DNA repair

DNA REPLICATION

Genetic information is transmitted from parent to progeny by replication of parental DNA, a process in which 2 daughter DNA molecules are produced that are each identical to the parental DNA molecule. During DNA replication, the 2 complementary strands of parental DNA are pulled apart. Each parental strand is then used as a template for the synthesis of a new complementary strand (semiconservative replication). During cell division, each daughter cell receives one of the 2 identical DNA molecules.

Replication of Prokaryotic and Eukaryotic Chromosomes

The process of DNA replication in prokaryotes and eukaryotes is compared below.

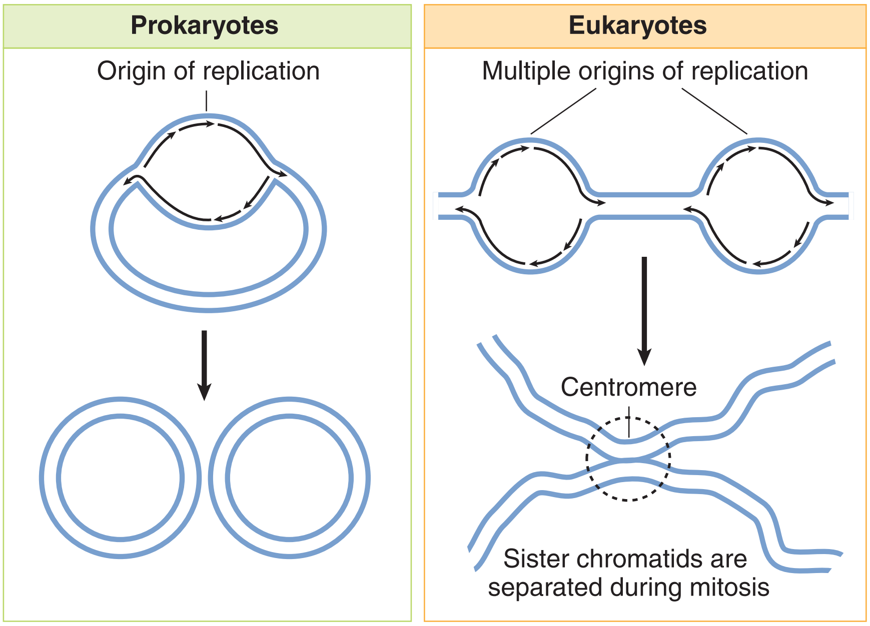


Figure I-2-1. DNA Replication by a Semi-Conservative, Bidirectional Mechanism

The bacterial chromosome is a closed, double-stranded circular DNA molecule having a single origin of replication. Separation of the 2 parental strands of DNA creates 2 replication forks that move away from each other in opposite directions around the circle. Replication is, thus, a bidirectional process. The 2 replication forks eventually meet, resulting in the production of 2 identical circular molecules of DNA.

Each eukaryotic chromosome contains one linear molecule of dsDNA having multiple origins of replication. Bidirectional replication occurs by means of a pair of replication forks produced at each origin. Completion of the process results in the production of 2 identical linear molecules of dsDNA (sister chromatids). DNA replication occurs in the nucleus during the S phase of the eukaryotic cell cycle. The 2 identical sister chromatids are separated from each other when the cell divides during mitosis.

Note

- **Polymerases** are enzymes that synthesize nucleic acids by forming phosphodiester (PDE) bonds.
- **Nucleases** are enzymes that hydrolyze PDE bonds.
  - Exonucleases remove nucleotides from the 5′ or the 3′ end of a nucleic acid.
  - Endonucleases cut within the nucleic acid and release nucleic acid fragments.

The structure of a representative eukaryotic chromosome during the cell cycle is shown below.

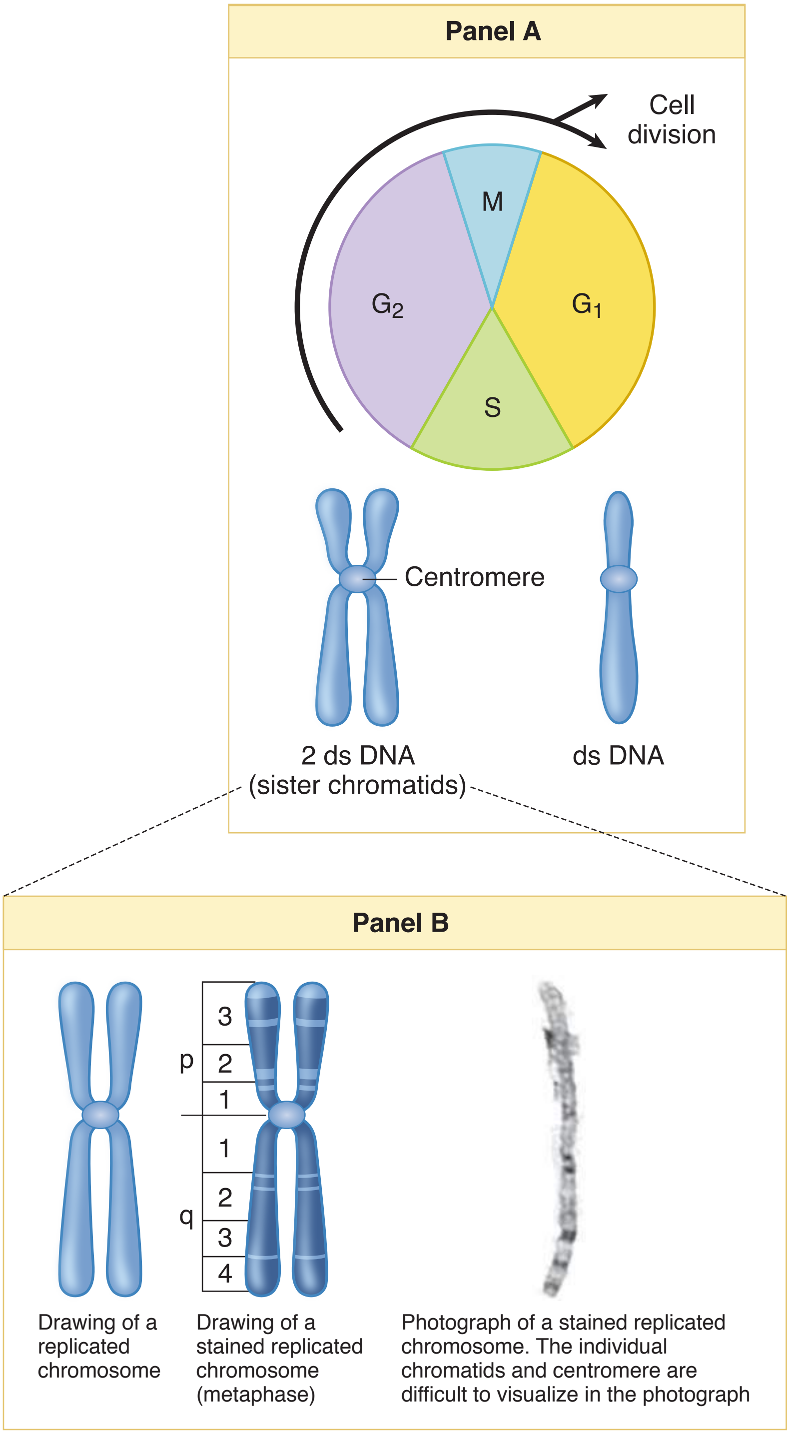


Figure I-2-2. Panel A: Eukaryotic Chromosome Replication During S-Phase
Panel B: Different Representations of a Replicated Eukaryotic Chromosome

COMPARISON OF DNA AND RNA SYNTHESIS

The overall process of DNA replication requires the synthesis of both DNA and RNA. These 2 types of nucleic acids are synthesized by DNA polymerases and RNA polymerases, respectively.

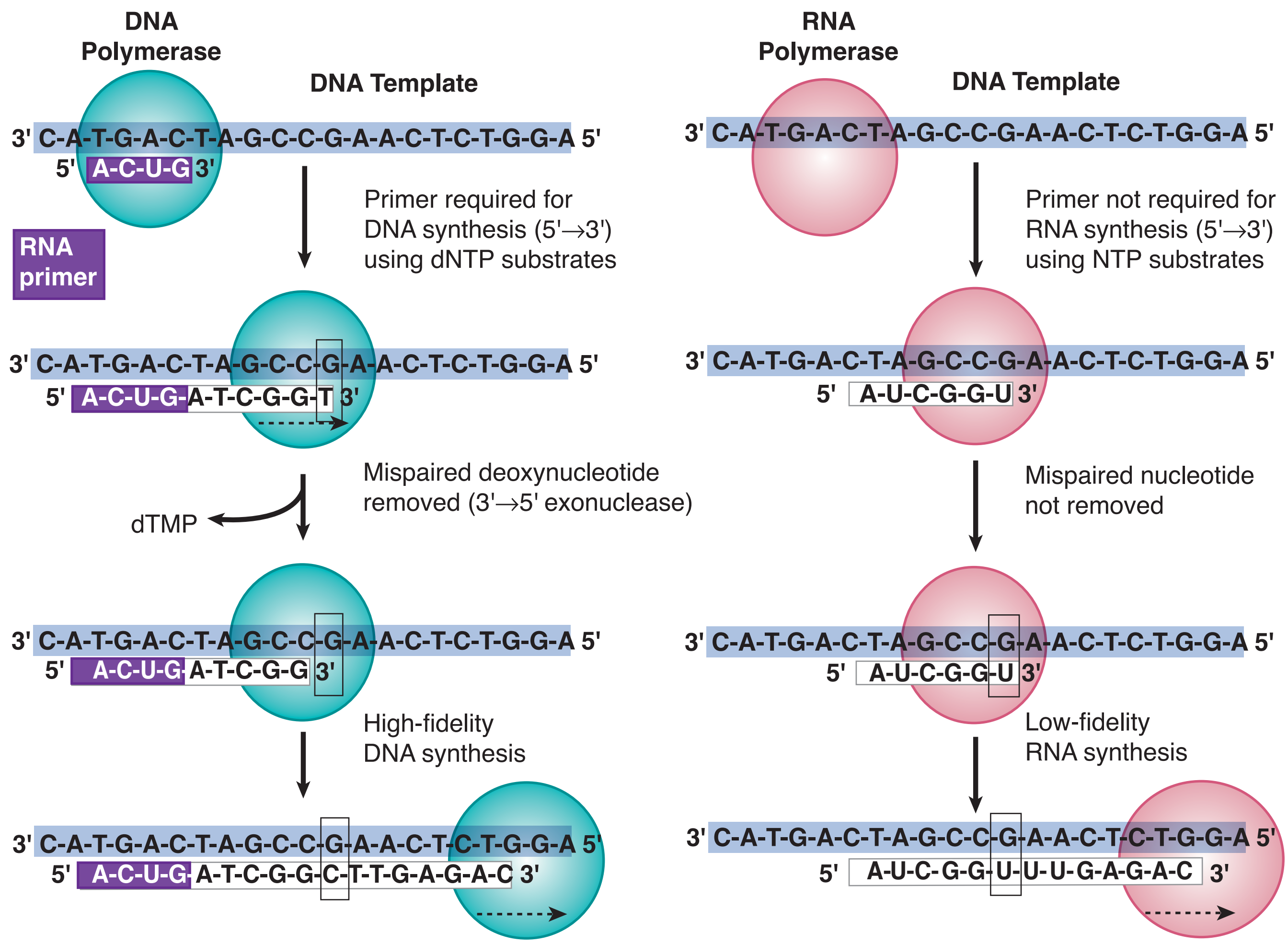


Figure I-2-3. Polymerase Enzymes Synthesize DNA and RNA

Table I-2-1. Comparison of DNA and RNA Polymerases

	DNA Polymerase	RNA Polymerase
Nucleic acid synthesized (5′→3′)	DNA	RNA
Required template (copied 3′→5′)	DNA*	DNA*
Required substrates	dATP, dGTP, dCTP, dTTP	ATP, GTP, CTP, UTP
Required primer	RNA (or DNA)	None
Proofreading activity (3′→5′ exonuclease)	Yes	No

* Certain DNA and RNA polymerases require RNA templates. These enzymes are most commonly associated with viruses.

Similarities between DNA and RNA synthesis include:

- The newly synthesized strand is made in the 5′→3′ direction.
- The template strand is scanned in the 3′→5′ direction.
- The newly synthesized strand is complementary and antiparallel to the template strand.
- Each new nucleotide is added when the 3′ hydroxyl group of the growing strand reacts with a nucleoside triphosphate, which is base-paired with the template strand. Pyrophosphate (PPi, the last two phosphates) is released during this reaction.

Differences include:

- The substrates for DNA synthesis are the dNTPs, whereas the substrates for RNA synthesis are the NTPs.
- DNA contains thymine, whereas RNA contains uracil.
- DNA polymerases require a primer, whereas RNA polymerases do not. That is, DNA polymerases cannot initiate strand synthesis, whereas RNA polymerases can.
- DNA polymerases can correct mistakes ("proofreading"), whereas RNA polymerases cannot. DNA polymerases have 3′ → 5′ exonuclease activity for proofreading.

STEPS OF DNA REPLICATION

The molecular mechanism of DNA replication is shown below. The sequence of events is as follows:

1. The base sequence at the origin of replication is recognized.
2. Helicase breaks the hydrogen bonds holding the base pairs together. This allows the two parental strands of DNA to begin unwinding and forms 2 replication forks.
3. Single-stranded DNA binding protein (SSB) binds to the single-stranded portion of each DNA strand, preventing them from reassociating and protecting them from degradation by nucleases.
4. Primase synthesizes a short (about 10 nucleotides) RNA primer in the 5′→3′ direction, beginning at the origin on each parental strand. The parental strand is used as a template for this process. RNA primers are required because DNA polymerases are unable to initiate synthesis of DNA, and can only extend a strand from the 3′ end of a preformed "primer."
5. DNA polymerase III begins synthesizing DNA in the 5′→3′ direction, beginning at the 3′ end of each RNA primer. The newly synthesized strand is complementary and antiparallel to the parental strand used as a template. This strand can be made continuously in one long piece and is known as the "leading strand."
    - The "lagging strand" is synthesized discontinuously as a series of small fragments (about 1,000 nucleotides long) known as Okazaki fragments. Each Okazaki fragment is initiated by the synthesis of an RNA primer by primase, and then completed by the synthesis of DNA using DNA polymerase III. Each fragment is made in the 5′→3′ direction.

- There is a leading and a lagging strand for each of the two replication forks on the chromosome.

6. RNA primers are removed by RNAase H in eukaryotes and an uncharacterized DNA polymerase fills in the gap with DNA. In prokaryotes DNA polymerase I both removes the primer (5' exonuclease) and synthesizes new DNA, beginning at the 3′ end of the neighboring Okazaki fragment.
7. Both eukaryotic and prokaryotic DNA polymerases have the ability to "proofread" their work by means of a 3′→5′ exonuclease activity. If DNA polymerase makes a mistake during DNA synthesis, the resulting unpaired base at the 3′ end of the growing strand is removed before synthesis continues.
8. DNA ligase seals the "nicks" between Okazaki fragments, converting them to a continuous strand of DNA.
9. DNA gyrase (DNA topoisomerase II) provides a "swivel" in front of each replication fork. As helicase unwinds the DNA at the replication forks, the DNA ahead of it becomes overwound and positive supercoils form. DNA gyrase inserts negative supercoils by nicking both strands of DNA, passing the DNA strands through the nick, and then resealing both strands. Quinolones are a family of drugs that block the action of topoisomerases. Nalidixic acid kills bacteria by inhibiting DNA gyrase. Inhibitors of eukaryotic topoisomerase II (etoposide, teniposide) are becoming useful as anticancer agents.

The mechanism of replication in eukaryotes is believed to be very similar to this. However, the details have not yet been completely worked out. The steps and proteins involved in DNA replication in prokaryotes are compared with those used in eukaryotes in Table I-2-2.

Eukaryotic DNA Polymerases

- DNA α and δ work together to synthesize both the leading and lagging strands.
- DNA polymerase γ replicates mitochondrial DNA.
- DNA polymerases β and ε are thought to participate primarily in DNA repair. DNA polymerase ε may substitute for DNA polymerase δ in certain cases.

Telomerase

High-Yield

Telomeres are repetitive sequences at the ends of linear DNA molecules in eukaryotic chromosomes. With each round of replication in most normal cells, the telomeres are shortened because DNA polymerase cannot complete synthesis of the 5′ end of each strand. This contributes to the aging of cells, because eventually the telomeres become so short that the chromosomes cannot function properly and the cells die.

Telomerase is an enzyme in eukaryotes used to maintain the telomeres. It contains a short RNA template complementary to the DNA telomere sequence, as well as telomerase reverse transcriptase activity (hTRT). Telomerase is thus able to replace telomere sequences that would otherwise be lost during replication. Normally telomerase activity is present only in embryonic cells, germ (reproductive) cells, and stem cells, but not in somatic cells.

Note

Telomerase

- Completes the replication of the telomere sequences at both ends of a eukaryotic chromosome
- Present in embryonic cells, fetal cells, and certain adult stem cells; not present in adult somatic cells
- Inappropriately present in many cancer cells, contributing to their unlimited replication

Cancer cells often have relatively high levels of telomerase, preventing the telomeres from becoming shortened and contributing to the immortality of malignant cells.

Table I-2-2. Steps and Proteins Involved in DNA Replication

Step in Replication	Prokaryotic Cells	Eukaryotic Cells (Nuclei)
Origin of replication (ori)	One ori site per chromosome	Multiple ori sites per chromosome
Unwinding of DNA double helix	Helicase	Helicase
Stabilization of unwound template strands	Single-stranded DNA-binding protein (SSB)	Single-stranded DNA-binding protein (SSB)
Synthesis of RNA primers	Primase	Primase
Synthesis of DNA Leading strand Lagging strand (Okazaki fragments)	DNA polymerase III DNA polymerase III	DNA polymerases $\alpha + \delta$ DNA polymerases $\alpha + \delta$
Removal of RNA primers	DNA polymerase I ($5' \rightarrow 3'$ exonuclease)	RNase H ($5' \rightarrow 3'$ exonuclease)
Replacement of RNA with DNA	DNA polymerase I	DNA polymerase δ
Joining of Okazaki fragments	DNA ligase	DNA ligase
Removal of positive supercoils ahead of advancing replication forks	DNA topoisomerase II (DNA gyrase)	DNA topoisomerase II
Synthesis of telomeres	Not required	Telomerase

Reverse Transcriptase

High-Yield

Reverse transcriptase is an RNA-dependent DNA polymerase that requires an RNA template to direct the synthesis of new DNA. Retroviruses, most notably HIV, use this enzyme to replicate their RNA genomes. DNA synthesis by reverse transcriptase in retroviruses can be inhibited by AZT, ddC, and ddI.

Eukaryotic cells also contain reverse transcriptase activity:

- Associated with telomerase (hTRT)
- Encoded by retrotransposons (residual viral genomes permanently maintained in human DNA) that play a role in amplifying certain repetitive sequences in DNA (see Chapter 7)

Bridge to Pharmacology

Quinolones and fluoroquinolones inhibit **DNA gyrase** (prokaryotic topoisomerase II), preventing DNA replication and transcription. These drugs, which are most active against aerobic gram-negative bacteria, include:

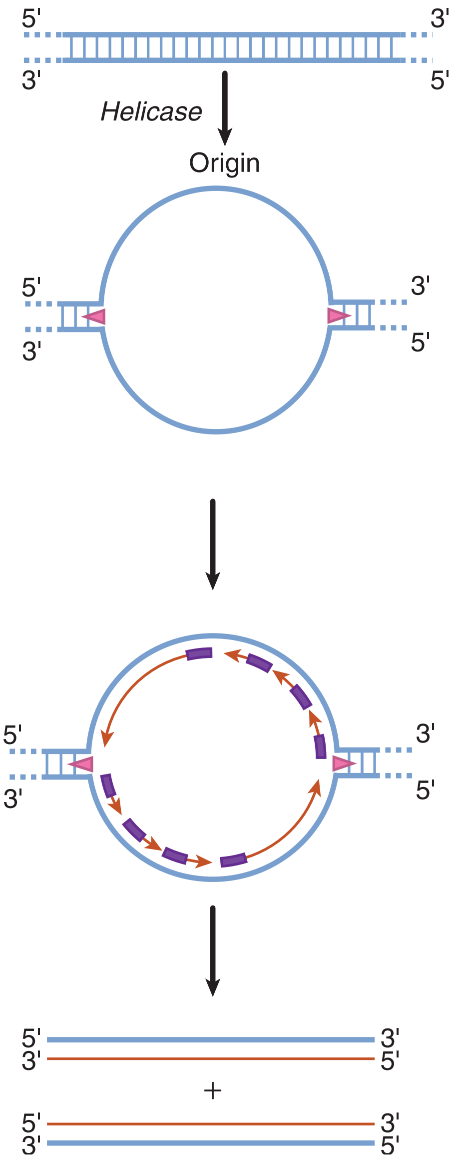
- Levofloxacin
- Ciprofloxacin
- Moxifloxacin

Resistance to the drugs has developed over time; current uses include treatment of gonorrhea and upper and lower urinary tract infections in both sexes.

Bridge to Pharmacology

One chemotherapeutic treatment of HIV is the use of AZT (3′-azido-2′,3′-dideoxythymidine) or structurally related compounds. Once AZT enters cells, it can be converted to the triphosphate derivative and used as a substrate for the viral reverse transcriptase in synthesizing DNA from its RNA genome.

The replacement of an azide instead of a normal hydroxyl group at the 3′ position of the deoxyribose prevents further replication by effectively causing chain termination. Although it is a DNA polymerase, reverse transcriptase lacks proofreading activity.



Leading Strand Synthesis (Continuous)

1. *Primase* synthesizes the primer (▬) 5' to 3'.
2. *DNA polymerases* α and δ extend the primer, moving **into** the replication fork (Leading strand synthesis).
3. *Helicase* (◄) continues to unwind the DNA.

Lagging Strand Synthesis (Discontinuous)

1. *Primase* synthesizes the primer (▬) 5' to 3'.
2. *DNA polymerases* α and δ extend the primer, moving **away from** the replication fork (Lagging strand synthesis).
3. Synthesis stops when *DNA polymerase* encounters the primer of the leading strand on the other side of the diagram (not shown), or the primer of the previous (Okazaki) fragment.
4. As *helicase* opens more of the replication fork, a third Okazaki fragment will be added.

RNase H (5' exoribonuclease activity) digests the RNA primer from fragment 1. In the eukaryotic cell, *DNA polymerase* extends the next fragment (2), to fill in the gap.

In prokaryotic cells *DNA polymerase 1* has both the 5' exonuclease activity to remove primers, and the *DNA polymerase* activity to extend the next fragment (2) to fill in the gap.

In both types of cells *DNA ligase* connects fragments 1 and 2 by making a phosphodiester bond.

This whole process repeats to remove all RNA primers from both the leading and lagging strands.

Figure I-2-4. DNA Replication

Recall Question

Which of the following enzymes is the target of fluoroquinolones in replication of DNA strands?

A. DNA gyrase

B. DNA helicase

C. DNA ligase

D. DNA polymerase

Answer: A

DNA REPAIR

The structure of DNA can be damaged in a number of ways through exposure to chemicals or radiation. Incorrect bases can also be incorporated during replication. Multiple repair systems have evolved, allowing cells to maintain the sequence stability of their genomes. If cells are allowed to replicate their DNA using a damaged template, there is a high risk of introducing stable mutations into the new DNA. Thus any defect in DNA repair carries an increased risk of cancer. Most DNA repair occurs in the G1 phase of the eukaryotic cell cycle. Mismatch repair occurs in the G2 phase to correct replication errors.

Table I-2-3. DNA Repair

Damage	Cause	Recognition/ Excision Enzyme	Repair Enzymes
Thymine dimers (G_1)	UV radiation	Excision endonuclease (deficient in Xeroderma pigmentosum)	DNA polymerase DNA ligase
Mismatched base (G_2)	DNA replication errors	A mutation on one of two genes, hMSH2 or hMLH1, initiates defective repair of DNA mismatches, resulting in a condition known as hereditary nonpolyposis colorectal cancer—HNPCC.	DNA polymerase DNA ligase
Cytosine deamination G_1	Spontaneous/ heat	Uracil glycosylase AP endonuclease	DNA polymerase DNA ligase

Repair of Thymine Dimers

High-Yield

Ultraviolet light induces the formation of dimers between adjacent thymines in DNA (also occasionally between other adjacent pyrimidines). The formation of thymine dimers interferes with DNA replication and normal gene expression. Thymine dimers are eliminated from DNA by a nucleotide excision-repair mechanism.

Bridge to Pathology

DNA repair may not occur properly when certain **tumor suppressor genes** have been inactivated through mutation or deletion:

- The *p53* gene encodes a protein that prevents a cell with damaged DNA from entering the S phase. Inactivation or deletion associated with Li Fraumeni syndrome and many solid tumors.
- *ATM* gene encodes a kinase essential for p53 activity. *ATM* is inactivated in ataxia telangiectasia, characterized by hypersensitivity to x-rays and predisposition to lymphomas. *BRCA-1* (breast, prostate, and ovarian cancer) and *BRCA-2* (breast cancer).
- The retinoblastoma *Rb* gene was the first tumor suppressor gene cloned, and is a negative regulator of the cell cycle through its ability to bind the transcription factor E2F and repress transcription of genes required for S phase.

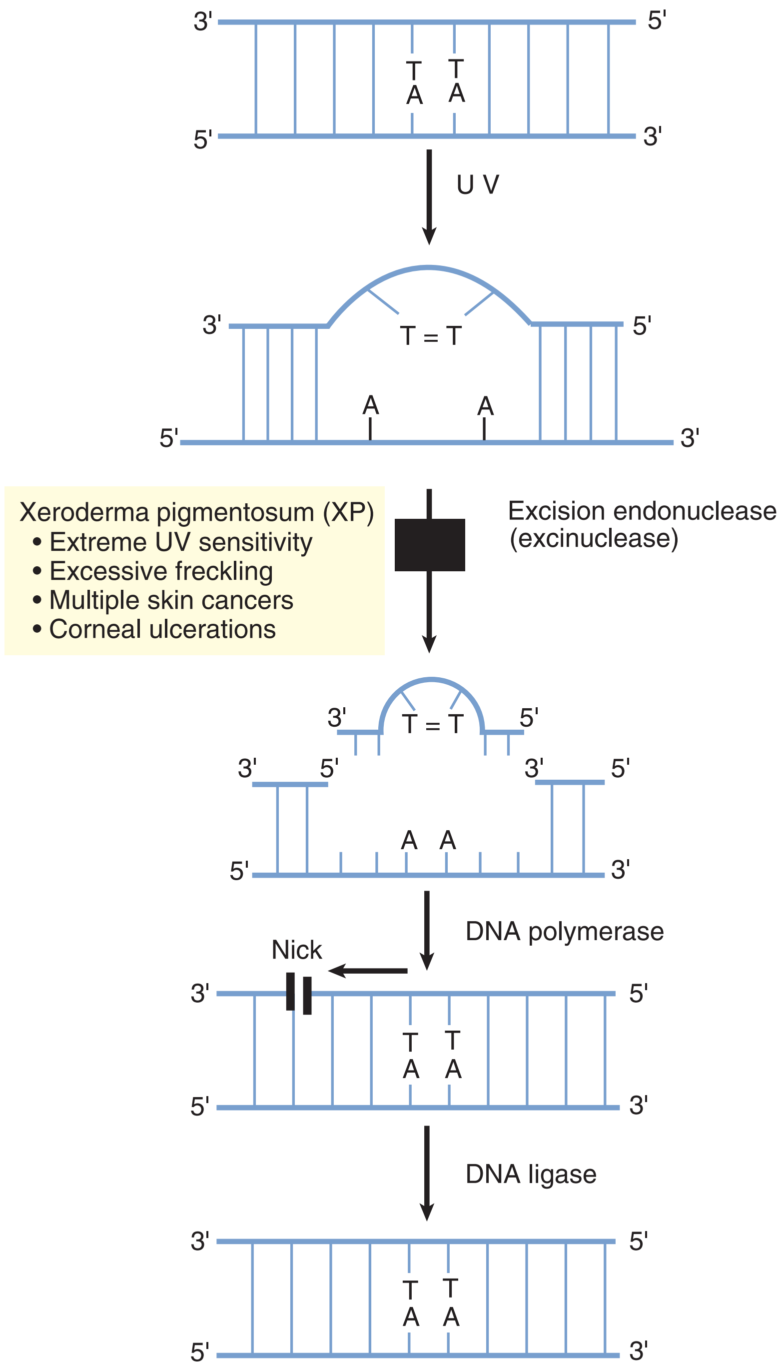


Figure I-2-5. Thymine Dimer Formation and Excision Repair

Steps in nucleotide excision repair:

- An excision endonuclease (excinuclease) makes nicks in the phosphodiester backbone of the damaged strand on both sides of the thymine dimer and removes the defective oligonucleotide.
- DNA polymerase fills in the gap by synthesizing DNA in the 5′→3′ direction, using the undamaged strand as a template.
- DNA ligase seals the nick in the repaired strand.

Base excision repair: cytosine deamination

Cytosine deamination (loss of an amino group from cytosine) converts cytosine to uracil. The uracil is recognized and removed (base excision) by a uracil glycosylase enzyme.

- Subsequently this area is recognized by an AP endonuclease that removes the damaged sequence from the DNA
- DNA polymerase fills in the gap
- DNA ligase seals the nick in the repaired strand

A summary of important genes involved in maintaining DNA fidelity and where they function in the cell cycle is shown below.

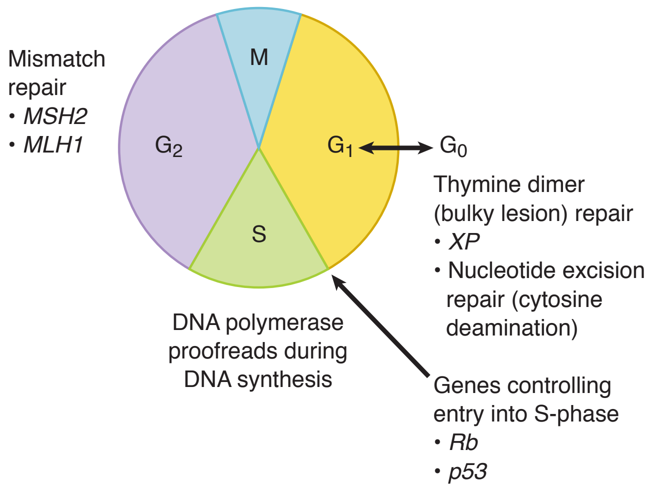


Figure I-2-6. Important Genes Associated with Maintaining Fidelity of Replicating DNA

Diseases Associated with DNA Repair

High-Yield

Inherited mutations that result in defective DNA repair mechanisms are associated with a predisposition to the development of cancer.

Xeroderma pigmentosum is an autosomal recessive disorder, characterized by extreme sensitivity to sunlight, skin freckling and ulcerations, and skin cancer. The most common deficiency occurs in the excinuclease enzyme.

Hereditary nonpolyposis colorectal cancer results from a deficiency in the ability to repair mismatched base pairs in DNA that are accidentally introduced during replication.

Xeroderma pigmentosum

Xeroderma pigmentosum is an autosomal recessive disorder (incidence 1/250,000) characterized by extreme sensitivity to sunlight, skin freckling, ulcerations, and skin cancer. Carcinomas and melanomas appear early in life, and most patients die of cancer. The most common deficiency occurs in the excision endonuclease.

> A 6-year-old child was brought to the clinic because his parents were concerned with excessive lesions and blistering in the facial and neck area. The parents noted that the lesions did not go away with typical ointments and creams and often became worse when the child was exposed to sunlight. The physician noted excessive freckling throughout the child's body, as well as slight stature and poor muscle tone.

Xeroderma pigmentosum can be diagnosed by measurement of the relevant enzyme excision endonuclease in white cells of blood. Patients with the disease should avoid exposure to any source of UV light.

Hereditary nonpolyposis colorectal cancer (Lynch syndrome)

Hereditary nonpolyposis colorectal cancer (HNPCC) results from a mutation in one of the genes (usually *hMLH1* or *hMSH2*) encoding enzymes that carry out DNA mismatch repair. These enzymes detect and remove errors introduced into the DNA during replication. In families with HNPCC, individuals may inherit one nonfunctional, deleted copy of the *hMLH1* gene or one nonfunctional, deleted copy of the *hMSH2* gene. After birth, a somatic mutation in the other copy may occur, causing loss of the mismatch repair function. This causes chromosomes to retain errors (mutations) in many other loci, some of which may contribute to cancer progression. This is manifested in intestinal cells because they are constantly undergoing cell division.

One prominent type of error that accompanies DNA replication is **microsatellite instability**. In a patient with HNPCC, cells from the resected tumor show microsatellite instability, whereas normal cells from the individual (which still retain mismatch repair) do not show microsatellite instability. Along with information from a family pedigree and histologic analysis, microsatellite instability may be used as a diagnostic tool.

Note

Microsatellites (also known as short tandem repeats) are di-, tri-, and tetranucleotide repeats dispersed throughout the DNA, usually (but not exclusively) in noncoding regions.

For example, TGTGTGTG may occur at a particular locus. If cells lack mismatch repair, the replicated DNA will vary in the number of repeats at that locus, e.g., TGTGTGTGTGTG or TGTGTG. This variation is **microsatellite instability**.

Review Questions

Select the ONE best answer.

1. It is now believed that a substantial proportion of the single nucleotide substitutions causing human genetic disease are due to misincorporation of bases during DNA replication. Which proofreading activity is critical in determining the accuracy of nuclear DNA replication and thus the base substitution mutation rate in human chromosomes?

    A. 3′ to 5′ polymerase activity of DNA polymerase δ

    B. 3′ to 5′ exonuclease activity of DNA polymerase γ

    C. Primase activity of DNA polymerase α

    D. 5′ to 3′ polymerase activity of DNA polymerase III

    E. 3′ to 5′ exonuclease activity of DNA polymerase δ

2. The proliferation of cytotoxic T-cells is markedly impaired upon infection with a newly discovered human immunodeficiency virus, designated HIV-V. The defect has been traced to the expression of a viral-encoded enzyme that inactivates a host-cell nuclear protein required for DNA replication. Which protein is a potential substrate for the viral enzyme?

    A. TATA-box binding protein (TBP)

    B. Cap binding protein (CBP)

    C. Catabolite activator protein (CAP)

    D. Acyl-carrier protein (ACP)

    E. Single-strand binding protein (SBP)

3. The deficiency of an excision endonuclease may produce an exquisite sensitivity to ultraviolet radiation in xeroderma pigmentosum. Which of the following functions would be absent in a patient deficient in this endonuclease?

    A. Removal of introns

    B. Removal of pyrimidine dimers

    C. Protection against DNA viruses

    D. Repair of mismatched bases during DNA replication

    E. Repair of mismatched bases during transcription

4. The anti-*Pseudomonas* action of norfloxacin is related to its ability to inhibit chromosome duplication in rapidly dividing cells. Which of the following enzymes participates in bacterial DNA replication and is directly inhibited by this antibiotic?

    A. DNA polymerase I

    B. DNA polymerase II

    C. Topoisomerase I

    D. Topoisomerase II

    E. DNA ligase

5. Cytosine arabinoside (araC) is used as an effective chemotherapeutic agent for cancer, although resistance to this drug may eventually develop. In certain cases, resistance is related to an increase in the enzyme cytidine deaminase in the tumor cells. This enzyme would inactivate araC to form

    A. cytosine
    B. cytidylic acid
    C. thymidine arabinoside
    D. uracil arabinoside
    E. cytidine

6. Dyskeratosis congenital (DKC) is a genetically inherited disease in which the proliferative capacity of stem cells is markedly impaired. The defect has been traced to inadequate production of an enzyme needed for chromosome duplication in the nuclei of rapidly dividing cells. Structural analysis has shown that the active site of this protein contains a single-stranded RNA that is required for normal catalytic function. Which step in DNA replication is most likely deficient in DKC patients?

    A. Synthesis of centromeres
    B. Synthesis of Okazaki fragments
    C. Synthesis of RNA primers
    D. Synthesis of telomeres
    E. Removal of RNA primers

7. Single-strand breaks in DNA comprise the single most frequent type of DNA damage. These breaks are frequently due to reactive oxygen species damaging the deoxyribose residues of the sugar phosphate backbone. This type of break is repaired by a series of enzymes that reconstruct the sugar and ultimately reform the phosphodiester bonds between nucleotides. Which class of enzyme catalyses the formation of the phosphodiester bond in DNA repair?

    A. DNA glycosylases
    B. DNA helicases
    C. DNA ligases
    D. DNA phosphodiesterases
    E. DNA polymerases

Answers

1. **Answer: E.** The 3′ to 5′ exonuclease activity of DNA pol δ represents the proofreading activity of an enzyme required for the replication of human chromosomal DNA. DNA pol γ (mitochondrial) and DNA pol III (prokaryotic) do not participate in this process, short RNA primers are replaced with DNA during replication, and new DNA strands are always synthesized in the 5′ to 3′ direction.

2. **Answer: E.** TBP and CBP participate in eukaryotic gene transcription and mRNA translation, respectively. CAP regulates the expression of prokaryotic lactose operons. ACP is involved in fatty acid synthesis.

3. **Answer: B.** Nucleotide excision repair of thymine (pyrimidine) dimers is deficient in XP patients.

4. **Answer: D.** Norfloxacin inhibits DNA gyrase (topoisomerase II).

5. **Answer: D.** Deamination of cytosine would produce uracil.

6. **Answer: D.** The enzyme is described as an RNA dependent DNA polymerase required for chromosome duplication in the nuclei of rapidly dividing cells. This enzyme is telomerase, a reverse transcriptase, that replicates the ends (telomeres) of linear chromosomes.

   None of the other options have reverse transcriptase activity.

7. **Answer: C.** All DNA repair systems use a ligase to seal breaks in the sugar phosphate backbone of DNA. Although polymerase enzymes make phosphodiester bonds during DNA synthesis, these enzymes do not ligate strands of DNA.

Transcription and RNA Processing 3

Learning Objectives

- ❏ Use knowledge of types of RNA
- ❏ Understand concepts of prokaryotic messenger RNA
- ❏ Understand concepts of eukaryotic messenger RNA
- ❏ Demonstrate understanding of alternative splicing of eukaryotic primary pre-mRNA transcripts
- ❏ Know key features of ribosomal RNA (rRNA)
- ❏ Know key features of transfer RNA (tRNA)

TRANSCRIPTION

The first stage in the expression of genetic information is transcription of the information in the base sequence of a double-stranded DNA molecule to form the base sequence of a single-stranded molecule of RNA. For any particular gene, only one strand of the DNA molecule (the template strand) is copied by RNA polymerase as it synthesizes RNA in the 5′ to 3′ direction. Because RNA polymerase moves in the 3′ to 5′ direction along the template strand of DNA, the RNA product is antiparallel and complementary to the template. RNA polymerase recognizes start signals (promoters) and stop signals (terminators) for each of the thousands of transcription units in the genome of an organism.

The figure below illustrates the arrangement and direction of transcription for several genes on a DNA molecule.

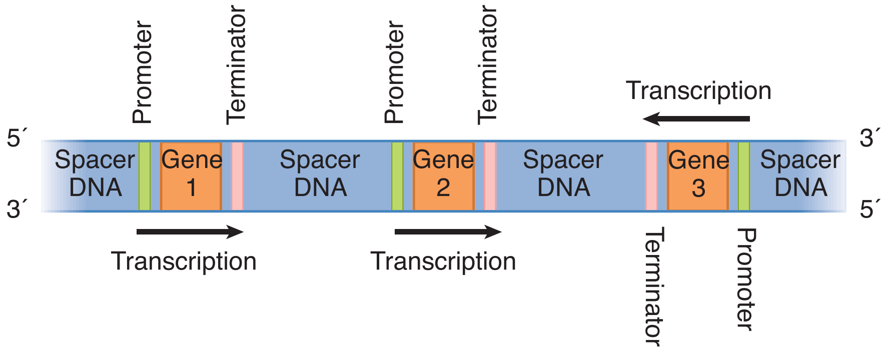


Figure I-3-1. Transcription of Several Genes on a Chromosome

TYPES OF RNA

RNA molecules play a variety of roles in the cell. The major types of RNA are:

- Ribosomal RNA (rRNA), which is the most abundant type of RNA in the cell. It is used as a structural component of the ribosome. Ribosomal RNA associates with ribosomal proteins to form the complete, functional ribosome.
- Transfer RNA (tRNA), which is the second most abundant type of RNA. Its function is to carry amino acids to the ribosome, where they will be linked together during protein synthesis.
- Messenger RNA (mRNA), which carries the information specifying the amino acid sequence of a protein to the ribosome. Messenger RNA is the only type of RNA that is translated. The mRNA population in a cell is very heterogeneous in size and base sequence, as the cell has essentially a different mRNA molecule for each of the thousands of different proteins made by that cell.
- Heterogeneous nuclear RNA (hnRNA or pre-mRNA), which is found only in the nucleus of eukaryotic cells. It represents precursors of mRNA, formed during its posttranscriptional processing.
- Small nuclear RNA (snRNA), which is also only found in the nucleus of eukaryotes. One of its major functions is to participate in splicing (removal of introns) mRNA.
- Ribozymes, which are RNA molecules with enzymatic activity. They are found in both prokaryotes and eukaryotes.

RNA POLYMERASES

There is a single prokaryotic RNA polymerase that synthesizes all types of RNA in the cell. The core polymerase responsible for making the RNA molecule has the subunit structure $\alpha_2\beta\beta'$. A protein factor called sigma (σ) is required for the initiation of transcription at a promoter. Sigma factor is released immediately after initiation of transcription. Termination of transcription sometimes requires a protein called rho (ρ) factor. The prokaryotic RNA polymerase is inhibited by rifampin. Actinomycin D binds to the DNA, preventing transcription.

There are 3 eukaryotic RNA polymerases, distinguished by the particular types of RNA they produce.

- RNA polymerase I is located in the nucleolus and synthesizes 28S, 18S, and 5.8S rRNAs.
- RNA polymerase II is located in the nucleoplasm and synthesizes hnRNA/mRNA and some snRNA.
- RNA polymerase III is located in the nucleoplasm and synthesizes tRNA, some snRNA, and 5S rRNA.

Transcription factors (such as TFIID for RNA polymerase II) help to initiate transcription. The requirements for termination of transcription in eukaryotes are not well understood. All transcription can be inhibited by actinomycin D. In addition, RNA polymerase II is inhibited by α-amanitin (a toxin from certain mushrooms).

Table I-3-1. Comparison of RNA Polymerases

Prokaryotic	Eukaryotic
Single RNA polymerase (α_2 ββ')	RNAP 1: rRNA (nucleolus) Except 5S rRNA RNAP 2: hnRNA/mRNA and some snRNA RNAP 3: tRNA, 5S rRNA
Requires sigma (σ) to initiate at a promoter	No sigma, but transcription factors (TFIID) bind before RNA polymerase
Sometimes requires rho (ρ) to terminate	No rho required
Inhibited by rifampin Actinomycin D	RNAP 2 inhibited by α-amanitin (mushrooms) Actinomycin D

TRANSCRIPTION: IMPORTANT CONCEPTS AND TERMINOLOGY

RNA is synthesized by a DNA-dependent RNA polymerase (uses DNA as a template for the synthesis of RNA). Important terminology used when discussing transcription is illustrated below.

- RNA polymerase locates genes in DNA by searching for promoter regions. The promoter is the binding site for RNA polymerase. Binding establishes where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds. No primer is required.
- RNA polymerase moves along the template strand in the 3′ to 5′ direction as it synthesizes the RNA product in the 5′ to 3′ direction using NTPs (ATP, GTP, CTP, UTP) as substrates. RNA polymerase does not proofread its work. The RNA product is complementary and antiparallel to the template strand.
- The coding (antitemplate) strand is not used during transcription. It is identical in sequence to the RNA molecule, except that RNA contains uracil instead of the thymine found in DNA.
- By convention, the base sequence of a gene is given from the coding strand (5′→3′).
- In the vicinity of a gene, a numbering system is used to identify the location of important bases. The first base transcribed as RNA is defined as the +1 base of that gene region.
  - To the left (5′, or upstream) of this starting point for transcription, bases are –1, –2, –3, etc.
  - To the right (3′, or downstream) of this point, bases are +2, +3, etc.
- Transcription ends when RNA polymerase reaches a termination signal.

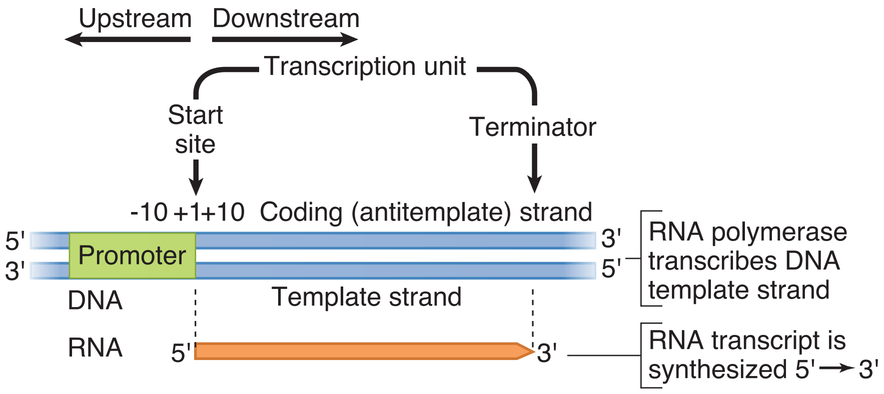


Figure I-3-2. Transcription of DNA

Flow of Genetic Information from DNA to Protein

High-Yield

For the case of a gene coding for a protein, the relationship among the sequences found in double-stranded DNA, single-stranded mRNA, and protein is illustrated below. Messenger RNA is synthesized in the 5′ to 3′ direction. It is complementary and antiparallel to the template strand of DNA. The ribosome translates the mRNA in the 5′ to 3′ direction, as it synthesizes the protein from the amino to the carboxyl terminus.

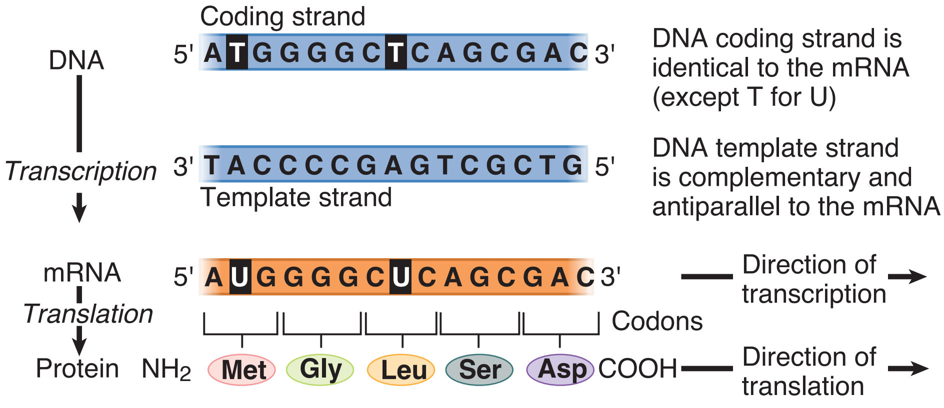


Figure I-3-3. Flow of Genetic Information from DNA to Protein

Sample Questions

1. During RNA synthesis, the DNA template sequence TAGC would be transcribed to produce which of the following sequences?

   A. ATCG

   B. GCTA

   C. CGTA

   D. AUCG

   E. GCUA

The answer is E. RNA is antiparallel and complementary to the template strand. Also remember that, by convention, all base sequences are written in the 5′ to 3′ direction regardless of the direction in which the sequence may actually be used in the cell.

Approach:

- Cross out any option with a T (RNA has U).
- Look at the 5′ end of DNA (T in this case).
- What is the complement of this base? (A)

Examine the options given. A correct option will have the complement (A in this example) at the 3′ end. Repeat the procedure for the 3′ end of the DNA. This will usually leave only one or two options.

2. Transcription of the following sequence of the tryptophan operon occurs in the direction indicated by the arrow. What would be the base sequence of the mRNA produced?

   3’ CGTCAGC 5’

   Transcription $\rightarrow$ Which product?

   5′…GCAGTCG…3′

   A. 5′…GCAGUCG…3′

   B. 5′…CGUGAGC…3′

   C. 5′…GCUGACG…3′

   D. 5′…CGUCAGC…3′

   E. 5′…CGUGAGC…3′

The answer is A. Because all nucleic acids are synthesized in the 5′ to 3′ direction, mRNA and the coding strand of DNA must each be oriented 5′ to 3′, i.e., in the direction of transcription. This means that the bottom strand in this example is the coding strand. The top strand is the template strand.

Approach:

- Cross out any option with a T.
- Identify the coding strand of DNA from the direction of transcription.
- Find the option with a sequence identical to the coding strand (remember to substitute U for T, if necessary).
- Alternatively, if you prefer to find the complement of the template strand, you will get the same answer.

PRODUCTION OF PROKARYOTIC MESSENGER RNA

The structure and expression of a typical prokaryotic gene coding for a protein are illustrated in Figure I-3-4. The following events occur during the expression of this gene:

1. With the help of sigma factor, RNA polymerase recognizes and binds to the promoter region. The bacterial promoter contains two "consensus" sequences, called the Pribnow box (or TATA box) and the –35 sequence. The promoter identifies the start site for transcription and orients the enzyme on the template strand. The RNA polymerase separates the two strands of DNA as it reads the base sequence of the template strand.
2. Transcription begins at the +1 base pair. Sigma factor is released as soon as transcription is initiated.
3. The core polymerase continues moving along the template strand in the 3′ to 5′ direction, synthesizing the mRNA in the 5′ to 3′ direction.
4. RNA polymerase eventually reaches a transcription termination signal, at which point it will stop transcription and release the completed mRNA molecule. There are two kinds of transcription terminators commonly found in prokaryotic genes:
    - Rho-independent termination occurs when the newly formed RNA folds back on itself to form a GC-rich hairpin loop closely followed by 6–8 U residues. These two structural features of the newly synthesized RNA promote dissociation of the RNA from the DNA template. This is the type of terminator shown in Figure I-3-4.
    - Rho-dependent termination requires participation of rho factor. This protein binds to the newly formed RNA and moves toward the RNA polymerase that has paused at a termination site. Rho then displaces RNA polymerase from the 3′ end of the RNA.
5. Transcription and translation can occur simultaneously in bacteria. Because there is no processing of prokaryotic mRNA (no introns), ribosomes can begin translating the message even before transcription is complete. Ribosomes bind to a sequence called the Shine-Dalgarno sequence in the 5′ untranslated region (UTR) of the message. Protein synthesis begins at an AUG codon at the beginning of the coding region and continues until the ribosome reaches a stop codon at the end of the coding region.
6. The ribosome translates the message in the 5′ to 3′ direction, synthesizing the protein from amino terminus to carboxyl terminus.

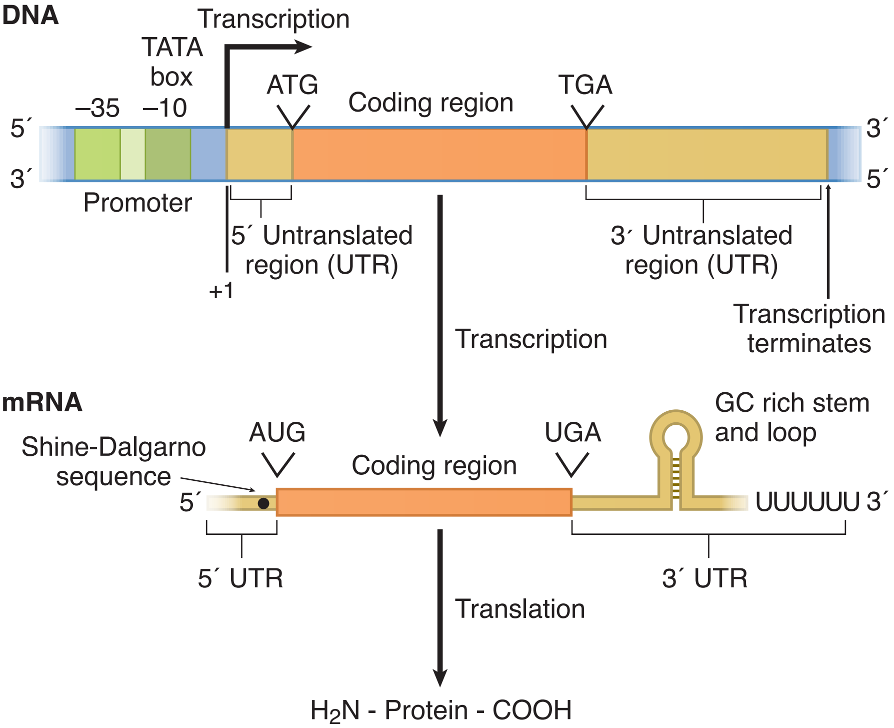


Figure I-3-4. Expression of a Prokaryotic Protein Coding Gene

The mRNA produced by the gene shown in the figure above is a monocistronic message. That is, it is transcribed from a single gene and codes for only a single protein. The word cistron is another name for a gene. Some bacterial operons (for example, the lactose operon) produce polycistronic messages. In these cases, related genes grouped together in the DNA are transcribed as one unit. The mRNA in this case contains information from several genes and codes for several different proteins.

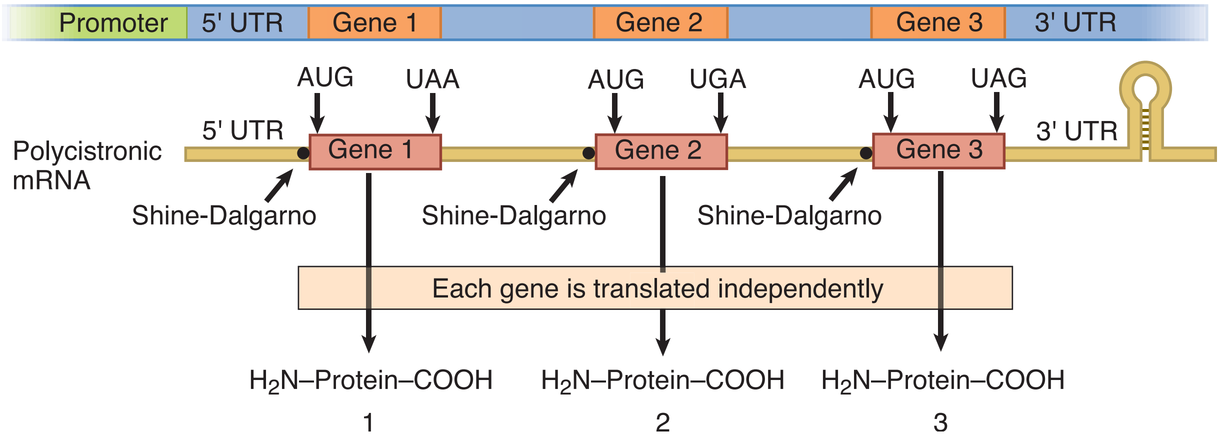


Figure I-3-5. Polycistronic Gene Region Codes for Several Different Proteins

PRODUCTION OF EUKARYOTIC MESSENGER RNA

In eukaryotes, most genes are composed of coding segments (exons) interrupted by noncoding segments (introns). Both exons and introns are transcribed in the nucleus. Introns are removed during processing of the RNA molecule in the nucleus. In eukaryotes, all mRNA is monocistronic. The mature mRNA is translated in the cytoplasm. The structure and transcription of a typical eukaryotic gene coding for a protein is illustrated in Figure I-3-6. Transcription of this gene occurs as follows:

1. With the help of proteins called transcription factors, RNA polymerase II recognizes and binds to the promoter region. The basal promoter region of eukaryotic genes usually has two consensus sequences called the TATA box (also called Hogness box) and the CAAT box.
2. RNA polymerase II separates the strands of the DNA over a short region to initiate transcription and read the DNA sequence. The template strand is read in the 3′ to 5′ direction as the RNA product (the primary transcript) is synthesized in the 5′ to 3′ direction. Both exons and introns are transcribed.
3. RNA polymerase II ends transcription when it reaches a termination signal. These signals are not well understood in eukaryotes.

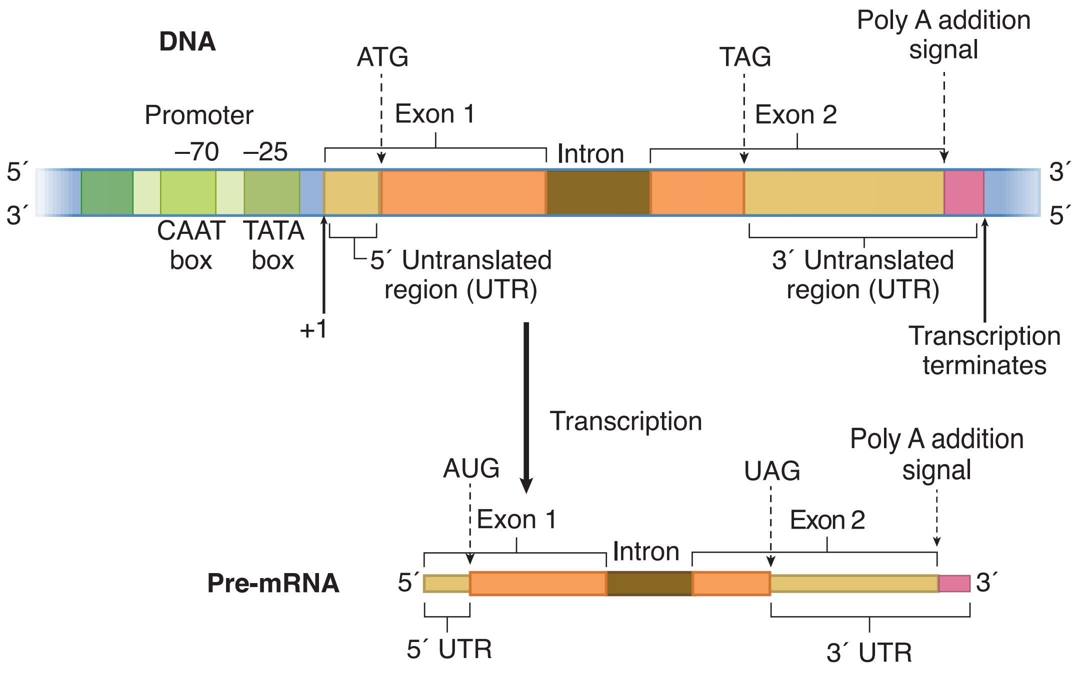


Figure I-3-6. A Eukaryotic Transcription Unit

Processing of Eukaryotic Pre-Messenger RNA

High-Yield

The primary transcript must undergo extensive posttranscriptional processing inside the nucleus to form the mature mRNA molecule. These processing steps include the following:

1. A 7-methylguanosine cap is added to the 5′ end while the RNA molecule is still being synthesized. The cap structure serves as a ribosome-binding site and also helps to protect the mRNA chain from degradation.

2. A poly-A tail is attached to the 3′ end. In this process, an endonuclease cuts the molecule on the 3′ side of the sequence AAUAAA (poly-A addition signal), then poly-A polymerase adds the poly-A tail (about 200 As) to the new 3′ end. The poly-A tail protects the message against rapid degradation and aids in its transport to the cytoplasm. A few mRNAs (for example, histone mRNAs) have no poly-A tails.

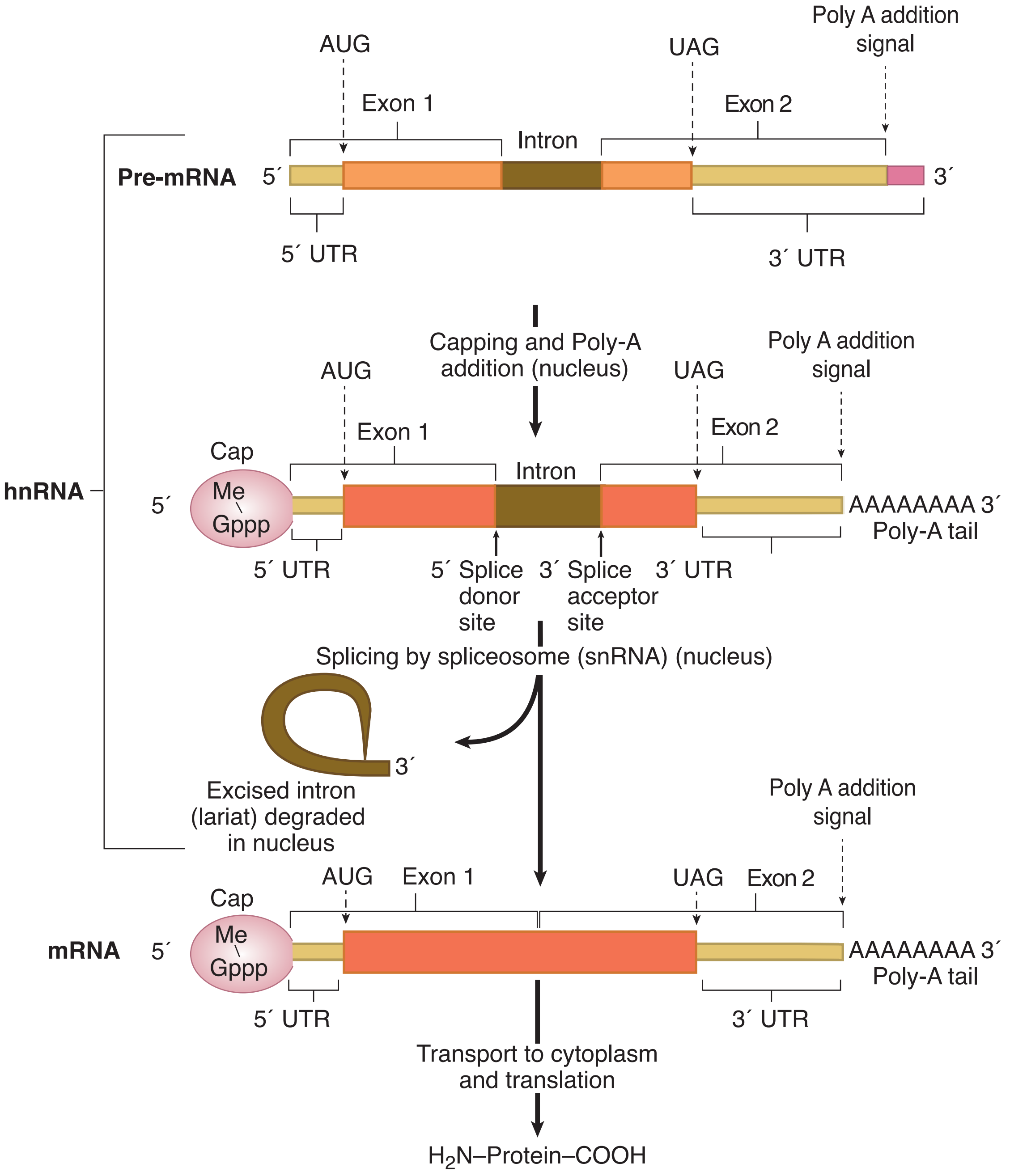


Figure I-3-7. Processing Eukaryotic Pre-mRNA

Note

Mutations in splice sites can lead to abnormal proteins. For example, mutations that interfere with proper splicing of β-globin mRNA are responsible for some cases of β-thalassemia.

3. Introns are removed from hnRNA by splicing, accomplished by spliceosomes (also known as an snRNP, or snurp), which are complexes of snRNA and protein. The hnRNA molecule is cut at splice sites at the 5′ (donor) and 3′ (acceptor) ends of the intron. The intron is excised in the form of a lariat structure and degraded. Neighboring exons are joined together to assemble the coding region of the mature mRNA.
4. All of the intermediates in this processing pathway are collectively known as hnRNA.
5. The mature mRNA molecule is transported to the cytoplasm, where it is translated to form a protein.

ALTERNATIVE SPLICING OF EUKARYOTIC PRIMARY PRE-mRNA TRANSCRIPTS

For some genes, the primary transcript is spliced differently to produce two or more variants of a protein from the same gene. This process is known as alternative splicing. Variants of the muscle proteins tropomyosin and troponin T are produced in this way. The synthesis of membrane-bound immunoglobulins by unstimulated B lymphocytes, as opposed to secreted immunoglobulins by antigen-stimulated B lymphocytes, also involves alternative splicing.

The primary transcripts from a large percentage of genes undergo alternative splicing. This may occur within the same cell, or the primary transcript of a gene may be alternatively spliced in different tissues, giving rise to tissue-specific protein products. By alternative splicing, an organism can make many more different proteins than it has genes to encode. A current estimate of the number of human proteins is at least 100,000, whereas the current estimate of human genes is about only 20,000–25,000. Alternative splicing can be detected by Northern blot, a technique discussed in Chapter 7.

Note

These figures should not be memorized because they may change upon more research.

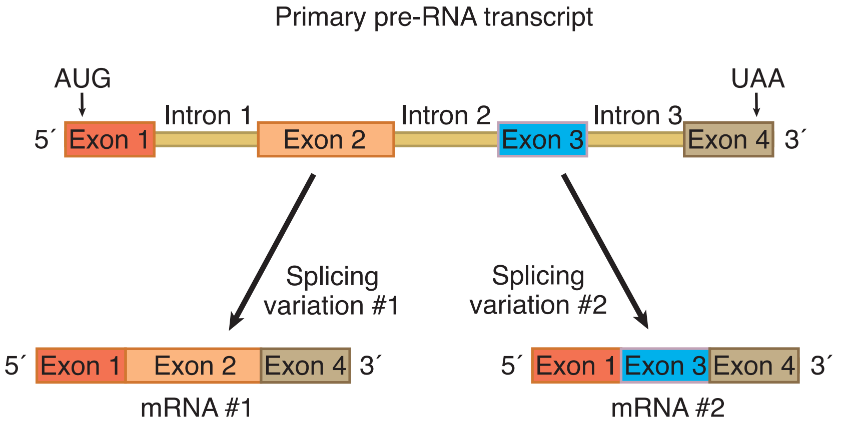


Figure I-3-8. Alternative Splicing of Eukaryotic hnRNA (Pre-mRNA) to Produce Different Proteins

RIBOSOMAL RNA (rRNA) IS USED TO CONSTRUCT RIBOSOMES

Eukaryotic ribosomal RNA is transcribed in the nucleolus by RNA polymerase I as a single piece of 45S RNA, which is subsequently cleaved to yield 28S rRNA, 18S rRNA, and 5.8S rRNA. RNA polymerase III transcribes the 5S rRNA unit from a separate gene. The ribosomal subunits assemble in the nucleolus as the rRNA pieces combine with ribosomal proteins. Eukaryotic ribosomal subunits are 60S and 40S. They join during protein synthesis to form the whole 80S ribosome.

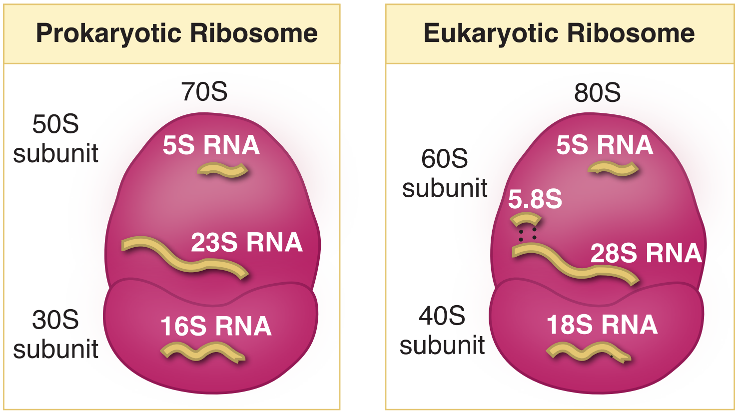


Figure I-3-9. The Composition of Prokaryotic and Eukaryotic Ribosomes

The large and small prokaryotic ribosomal subunits are 50S and 30S, respectively. The complete prokaryotic ribosome is a 70S particle. (Note: The S values are determined by behavior of the particles in an ultracentrifuge. They are a function of both size and shape, and therefore the numbers are not additive.)

TRANSFER RNA (tRNA) CARRIES ACTIVATED AMINO ACIDS FOR TRANSLATION

There are many different specific tRNAs. Each tRNA carries only one type of activated amino acid for making proteins during translation. The genes encoding these tRNAs in eukaryotic cells are transcribed by RNA polymerase III. The tRNAs enter the cytoplasm where they combine with their appropriate amino acids. Although all tRNAs have the same general shape shown in Figure I-3-10, small structural features distinguish among them.

Bridge to Microbiology

Shiga toxin (*Shigella dysenteriae*) and Verotoxin, a shiga-like toxin (enterohemorrhagic *E. coli*), inactivate the 28S rRNA in the 60S subunit of the eukaryotic ribosome. The A subunits of these toxins are RNA glycosylases that remove a single adenine residue from the 28S rRNA. This prevents aminoacyl-tRNA binding to the ribosome, halting protein synthesis.

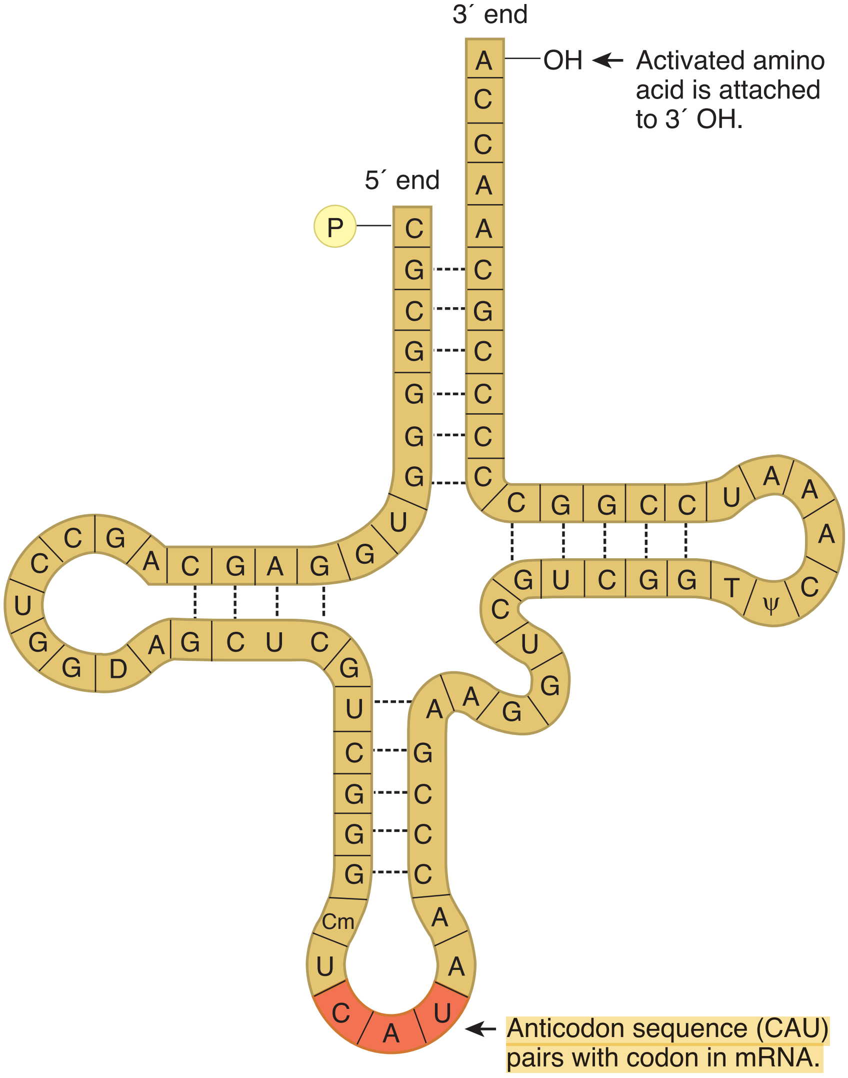


Figure I-3-10. Transfer RNA (tRNA)

RNA Editing

RNA editing is a process by which some cells make discrete changes to specific nucleotide sequences within a RNA molecule after its gene has been transcribed by RNA polymerase. Posttranscription editing events may include insertion, deletion, and base alterations of nucleotides (such as adenine deamination) within the edited RNA molecule. RNA editing has been observed in some mRNA, rRNA, and tRNA molecules in humans.

An important example is cytosine-to-uracil deamination in the apoprotein B gene. Apoprotein B100 is expressed in the liver, and apoprotein B48 is expressed in the intestines. In the intestines, the mRNA is edited from a CAA sequence to be UAA, a stop codon, thus producing the shorter apoprotein B48 form.

Recall Question

Which of the following is a co-transcriptional event in RNA synthesis?

A. Addition of a 7-methyl G cap

B. Splicing

C. Poly A tailing

Answer: A

Table I-3-2. Important Points About Transcription and RNA Processing

	Prokaryotic	Eukaryotic
Gene regions	May be polycistronic Genes are continuous coding regions Very little spacer (noncoding) DNA between genes	Always monocistronic Genes have exons and introns Large spacer (noncoding) DNA between genes
RNA polymerase	Core enzyme: $\alpha_2\beta\beta'$	RNA polymerase I: rRNA RNA polymerase II: mRNA; snRNA RNA polymerase III: tRNA, 5S RNA
Initiation of transcription	Promoter (–10) TATAAT and (–35) sequence Sigma initiation subunit required to recognize promoter	Promoter (–25) TATA and (–70) CAAT Transcription factors (TFIID) bind promoter
mRNA synthesis	Template read 3′ to 5′; mRNA synthesized 5′ to 3′; gene sequence specified from coding strand 5′ to 3′; transcription begins at +1 base	
Termination of transcription	Stem and loop + UUUUU Stem and loop + rho factor	Not well characterized
Relationship of RNA transcript to DNA	RNA is antiparallel and complementary to DNA template strand; RNA is identical (except U substitutes for T) to DNA coding strand	
Posttranscriptional processing of hnRNA (pre-mRNA)	None	In nucleus: 5′ cap (7-MeG) 3′ tail (poly-A sequence) Removal of introns from pre-RNA • Alternative splicing yields variants of protein product
Ribosomes	70S (30S and 50S) rRNA and protein	80S (40S and 60S) rRNA and protein
tRNA	Cloverleaf secondary structure • Acceptor arm (CCA) carries amino acid • Anticodon arm; anticodon complementary and antiparallel to codon in mRNA	

Review Questions

Select the ONE best answer.

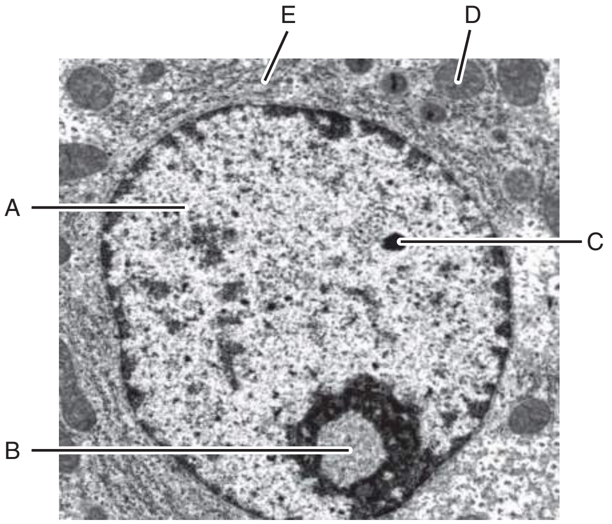
1. The base sequence of codons 57-58 in the cytochrome β5 reductase gene is CAGCGC. The mRNA produced upon transcription of this gene will contain which sequence?

   A. GCGCTG
   B. CUGCGC
   C. GCGCUG
   D. CAGCGC
   E. GUCGCG

2. A gene encodes a protein with 150 amino acids. There is one intron of 1,000 bps, a 5′-untranslated region of 100 bp, and a 3′-untranslated region of 200 bp. In the final processed mRNA, how many bases lie between the start AUG codon and the final termination codon?

   A. 1,750
   B. 750
   C. 650
   D. 450
   E. 150

Items 3–5: Identify the nuclear location.



3. Transcription of genes by RNA polymerase 1

4. Euchromatin

5. Polyadenylation of pre-mRNA by poly-A polymerase

Answers

1. **Answer: D.** Since the sequence in the stem represents the coding strand, the mRNA sequence must be identical (except U for T). No T in the DNA means no U in the mRNA.

2. **Answer: D.** Only the coding region remains to be calculated $3 \times 150 = 450$.

3. **Answer: B.** rRNA genes are transcribed by this enzyme in the nucleolus.

4. **Answer: A.** Less condensed chromatin, lighter areas in the nucleus. Darker areas are heterochromatin.

5. **Answer: A.** Polyadenylation of pre-mRNA occurs in the nucleoplasm. Generally associated with active gene expression in euchromatin.

The Genetic Code, Mutations, and Translation

4

Learning Objectives

- ❑ Demonstrate understanding of the genetic code
- ❑ Solve problems concerning mutations
- ❑ Interpret scenarios about amino acid activation and codon translation by tRNAs
- ❑ Demonstrate understanding of translation (protein synthesis)
- ❑ Explain information related to inhibitors of protein synthesis
- ❑ Interpret scenarios about protein folding and subunit assembly
- ❑ Answer questions about how translation occurs on the rough endoplasmic reticulum
- ❑ Demonstrate understanding of co- and posttranslational covalent modifications
- ❑ Solve problems concerning posttranslational modifications of collagen

TRANSLATION

The second stage in gene expression is translating the nucleotide sequence of a messenger RNA molecule into the amino acid sequence of a protein. The genetic code is defined as the relationship between the sequence of nucleotides in DNA (or its RNA transcripts) and the sequence of amino acids in a protein. Each amino acid is specified by one or more nucleotide triplets (codons) in the DNA.

During translation, mRNA acts as a working copy of the gene in which the codons for each amino acid in the protein have been transcribed from DNA to mRNA. tRNAs serve as adapter molecules that couple the codons in mRNA with the amino acids they each specify, thus aligning them in the appropriate sequence before peptide bond formation. Translation takes place on ribosomes, complexes of protein and rRNA that serve as the molecular machines coordinating the interactions between mRNA, tRNA, the enzymes, and the protein factors required for protein synthesis. Many proteins undergo posttranslational modifications as they prepare to assume their ultimate roles in the cell.

THE GENETIC CODE

Most genetic code tables designate the codons for amino acids as mRNA sequences. Important features of the genetic code include:

- Each codon consists of 3 bases (triplet). There are 64 codons. They are all written in the 5′ to 3′ direction.
- 61 codons code for amino acids. The other 3 (UAA, UGA, UAG) are stop codons (or nonsense codons) that terminate translation.
- There is one start codon (initiation codon), AUG, coding for methionine. Protein synthesis begins with methionine (Met) in eukaryotes, and formylmethionine (fMet) in prokaryotes.
- The code is unambiguous. Each codon specifies no more than one amino acid.
- The code is degenerate. More than one codon can specify a single amino acid. All amino acids, except Met and tryptophan (Trp), have more than one codon.
- For those amino acids having more than one codon, the first 2 bases in the codon are usually the same. The base in the third position often varies.
- The code is universal (the same in all organisms). Some minor exceptions to this occur in mitochondria.
- The code is commaless (contiguous). There are no spacers or “commas” between codons on an mRNA.
- Neighboring codons on a message are nonoverlapping.

First Position (5' End)	Second Position: U	C	A	G	Third Position (3' End)
U	UUU, UUC } Phe UUA, UUG } Leu	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr UAA, UAG } Stop	UGU, UGC } Cys UGA Stop UGG Trp	U C A G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U C A G
A	AUU, AUC, AUA } Ile AUG Met	ACU, ACC, ACA, ACG } Thr	AAU, AAC } Asn AAA, AAG } Lys	AGU, AGC } Ser AGA, AGG } Arg	U C A G
G	GUU, GUC, GUA, GUG } Val	GCU, GCC, GCA, GCG } Ala	GAU, GAC } Asp GAA, GAG } Glu	GGU, GGC, GGA, GGG } Gly	U C A G

Figure I-4-1. The Genetic Code

MUTATIONS

A mutation is any permanent, heritable change in the DNA base sequence of an organism. This altered DNA sequence can be reflected by changes in the base sequence of mRNA, and, sometimes, by changes in the amino acid sequence of a protein. Mutations can cause genetic diseases. They can also cause changes in enzyme activity, nutritional requirements, antibiotic susceptibility, morphology, antigenicity, and many other properties of cells.

A very common type of mutation is a single base alteration or point mutation.

- A transition is a point mutation that replaces a purine-pyrimidine base pair with a different purine-pyrimidine base pair. For example, an A-T base pair becomes a G-C base pair.
- A transversion is a point mutation that replaces a purine-pyrimidine base pair with a pyrimidine-purine base pair. For example, an A-T base pair becomes a T-A or a C-G base pair.

Mutations are often classified according to the effect they have on the structure of the gene's protein product. This change in protein structure can be predicted using the genetic code table in conjunction with the base sequence of DNA or mRNA. A variety of such mutations is listed in Table I-4-1. Point mutations and frameshifts are illustrated in more detail in Figure I-4-2.

Table I-4-1. Effects of Some Common Types of Mutations on Protein Structure

Type of Mutation	Effect on Protein
Silent: new codon specifies same amino acid	None
Missense: new codon specifies different amino acid	Possible decrease in function; variable effects
Nonsense: new codon is stop codon	Shorter than normal; usually nonfunctional
Frameshift/in-frame: addition or deletion of base(s)	Usually nonfunctional; often shorter than normal
Large segment deletion (unequal crossover in meiosis)	Loss of function; shorter than normal or entirely missing
5′ splice site (donor) or 3′ splice site (acceptor)	Variable effects ranging from addition or deletion of a few amino acids to deletion of an entire exon
Trinucleotide repeat expansion	Expansions in coding regions cause protein product to be longer than normal and unstable. Disease often shows anticipation in pedigree.

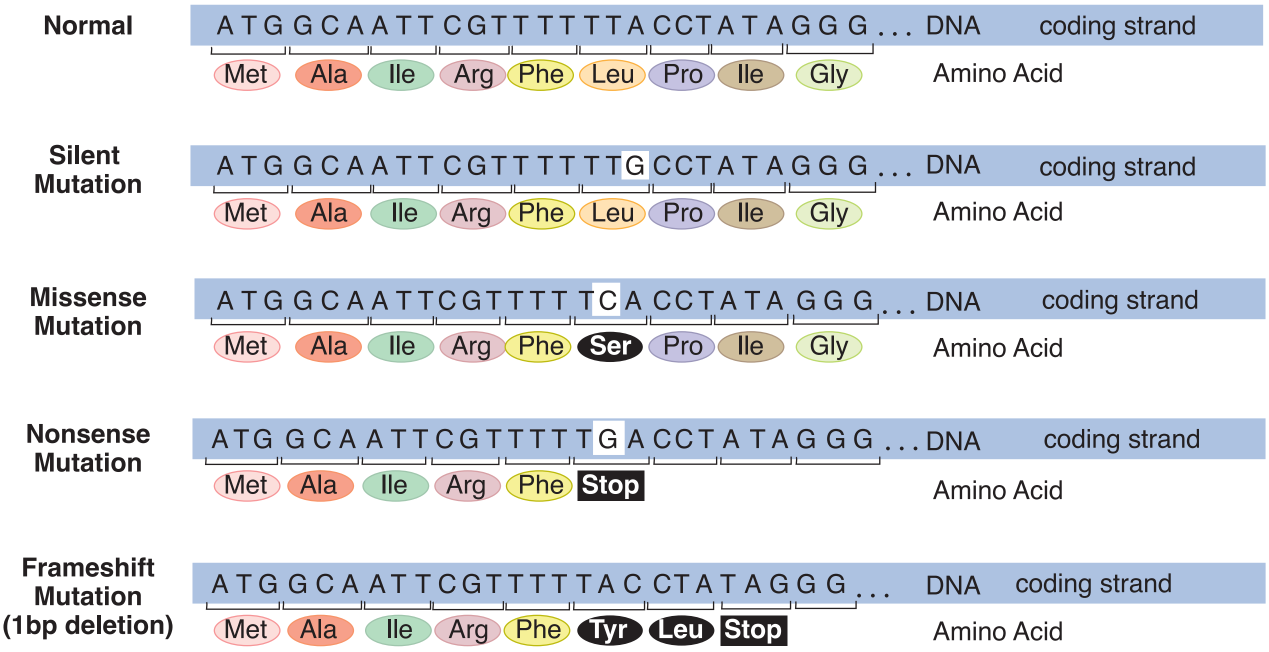


Figure I-4-2. Some Common Types of Mutations in DNA

Large Segment Deletions

Large segments of DNA can be deleted from a chromosome during an unequal crossover in meiosis. Crossover or recombination between homologous chromosomes is a normal part of meiosis I that generates genetic diversity in reproductive cells (egg and sperm), a largely beneficial result. In a normal crossover event, the homologous maternal and paternal chromosomes exchange equivalent segments, and although the resultant chromosomes are mosaics of maternal and paternal alleles, no genetic information has been lost from either one. On rare occasions, a crossover can be unequal and one of the two homologs loses some of its genetic information.

α-thalassemia is a well-known example of a genetic disease in which unequal crossover has deleted one or more α-globin genes from chromosome 16. Cri-du-chat (mental retardation, microcephaly, wide-set eyes, and a characteristic kitten-like cry) results from a terminal deletion of the short arm of chromosome 5.

Mutations in Splice Sites

High-Yield

Mutations in splice sites affect the accuracy of intron removal from hnRNA during posttranscriptional processing. If a splice site is lost through mutation, spliceosomes may:

- Delete nucleotides from the adjacent exon
- Leave nucleotides of the intron in the processed mRNA
- Use the next normal upstream or downstream splice site, deleting an exon from the processed mRNA

Mutations in splice sites have now been documented in many different diseases, including β-thalassemia, Gaucher disease, and Tay-Sachs.

β-Thalassemia

There are two genes for the beta chain of hemoglobin. In β-thalassemia, there is a deficiency of β-globin protein compared with α-globin. A large number of β-globin mutations have been described, including gene deletions, mutations that slow the transcriptional process, and translational defects involving nonsense and frameshift mutations. Other mutations involve β-globin mRNA processing (more than 70% of the β-globin gene is not encoding information and eventually must be spliced out), such as splice site mutations at the consensus sequences. Also, mutations within intron 1 create a new splice site, resulting in an abnormally long mRNA.

> A 9-month-old infant of Greek descent was brought to the hospital by his parents because he became pale, listless, and frequently irritable. The attending physician noted that the spleen was enlarged and that the infant was severely anemic. His face had "rat-like" features due to deformities in the skull.

β-thalassemias are found primarily in Mediterranean areas. It is believed that, similar to sickle cell anemia and glucose-6-phosphate dehydrogenase deficiency, the abnormality of red blood cells in β-thalassemia may protect against malaria. Splenomegaly is due to the role of the spleen in clearing damaged red cells from the bloodstream. The excessive activity of the bone marrow produces bone deformities of the face and other areas. The long bones of the arms and legs are abnormally weak and fracture easily. The most common treatment is blood transfusions every 2–3 weeks, but iron overload is a serious consequence.

Trinucleotide Repeat Expansion

High-Yield

The mutant alleles in certain diseases, such as Huntington disease, fragile X syndrome, and myotonic dystrophy, differ from their normal counterparts only in the number of tandem copies of a trinucleotide. In these diseases, the number of repeats often increases with successive generations and correlates with increasing severity and decreasing age of onset, a phenomenon called anticipation. For example, in the normal Huntington allele, there are 5 tandem repeats of CAG in the coding region. Affected family members may have 30–60 of these CAG repeats. The normal protein contains 5 adjacent glutamine residues, whereas the proteins encoded by the disease-associated alleles have 30 or more adjacent glutamines. The long glutamine tract makes the abnormal proteins extremely unstable. A major clinical manifestation of the trinucleotide repeat expansion disorders is neurodegeneration of specific neurons.

The expansion of the trinucleotide repeat in the mutant allele can be in a coding region or in an untranslated region of the gene.

Table I-4-2. Two Classes of Trinucleotide Repeat Expansion Diseases

Translation repeat disorders (polyglutamine disorders)	Untranslated repeat disorders
Huntington disease: (CAG)n	Fragile X syndrome: (CGG)n
Spinobulbar muscular atrophy: (CAG)n	Myotonic dystrophy: (CTG)n Friedreich's ataxia: (GAA)n

Clinical Correlate

Huntington disease, an autosomal dominant disorder, has a mean age-of-onset in decade 4. Symptoms appear gradually and worsen over about 15 years until death occurs. Mood disturbance, impaired memory, and hyperreflexia are often the first signs, followed by abnormal gait, chorea (loss of motor control), dystonia, dementia, and dysphagia.

Cases of juvenile onset (age <10) are more severe and most frequently occur when the defective allele is inherited paternally. About 25% of cases have late onset, slower progression, and milder symptoms.

AMINO ACID ACTIVATION AND CODON TRANSLATION BY tRNAs

Inasmuch as amino acids have no direct affinity for mRNA, an adapter molecule, which recognizes an amino acid on one end and its corresponding codon on the other, is required for translation. This adapter molecule is tRNA.

Amino Acid Activation

As tRNAs enter the cytoplasm, each combines with its cognate amino acid in a process called amino acid activation.

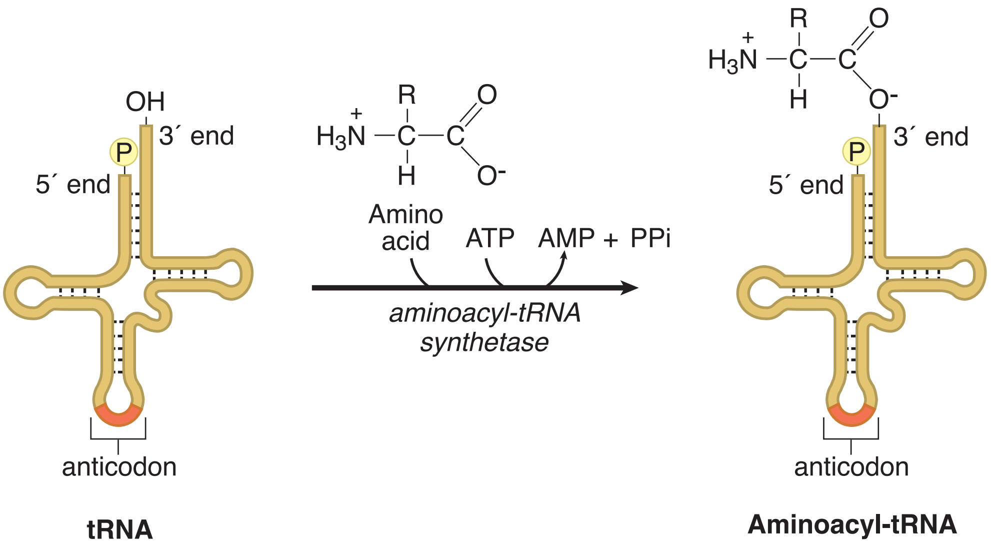


Figure I-4-3. Activation of Amino Acid for Protein Synthesis

- Each type of amino acid is activated by a different aminoacyl tRNA synthetase.
- Two high-energy bonds from an ATP are required.
- The aminoacyl tRNA synthetase transfers the activated amino acid to the 3´ end of the correct tRNA.
- The amino acid is linked to its cognate tRNA with an energy-rich bond.
- This bond will later supply energy to make a peptide bond linking the amino acid into a protein.

Aminoacyl tRNA synthetases have self-checking functions to prevent incorrectly paired aminoacyl tRNAs from forming. If, however, an aminoacyl tRNA synthetase does release an incorrectly paired product (ala-tRNA^{ser}), there is no mechanism during translation to detect the error and an incorrect amino acid will be introduced into some protein.

Each tRNA has an anticodon sequence that allows it to pair with the codon for its cognate amino acid in the mRNA. Because base pairing is involved, the orientation of this interaction will be complementary and antiparallel. For example, the amino acyl tRNA arg-tRNA^{arg} has an anticodon sequence, UCG, allowing it to pair with the arginine codon CGA.

TRANSLATION (PROTEIN SYNTHESIS)

Protein synthesis occurs by peptide bond formation between successive amino acids whose order is specified by a gene and thus by an mRNA. The formation of a peptide bond between the carboxyl group on one amino acid and the amino group of another is illustrated below.

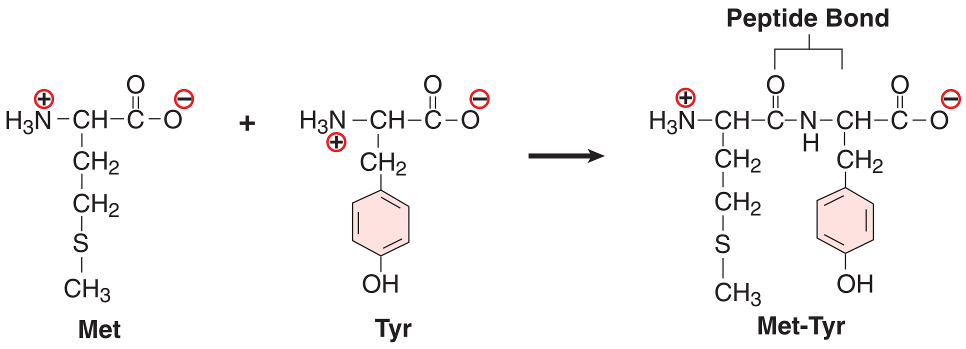


Figure I-4-4. Peptide Bond Formation

During translation, the amino acids are attached to the 3′ ends of their respective tRNAs. The aminoacyl–tRNAs are situated in the P and A sites of the ribosome as shown in the figure below. Notice that the peptide bond forms between the carboxyl group of the amino acid (or growing peptide) in the P site and the amino group of the next amino acid in the A site. Proteins are synthesized from the amino to the carboxyl terminus.

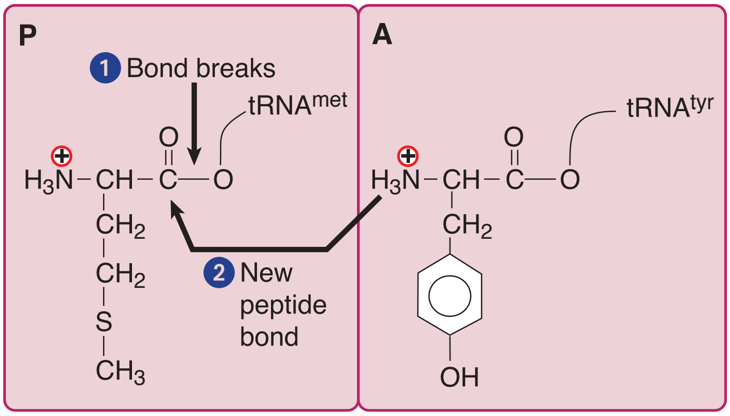


Figure I-4-5. Formation of a Peptide Bond by a Ribosome During Translation

Steps of Translation

High-Yield

Translation occurs in the cytoplasm of both prokaryotic (Pr) and eukaryotic (Eu) cells. In prokaryotes, ribosomes can begin translating the mRNA even before RNA polymerase completes its transcription. In eukaryotes, translation and transcription are completely separated in time and space with transcription

in the nucleus and translation in the cytoplasm. The process of protein synthesis occurs in 3 stages: initiation, elongation, and termination.

Special protein factors for initiation (IF), elongation (EF), and termination (release factors), as well as GTP, are required for each stage.

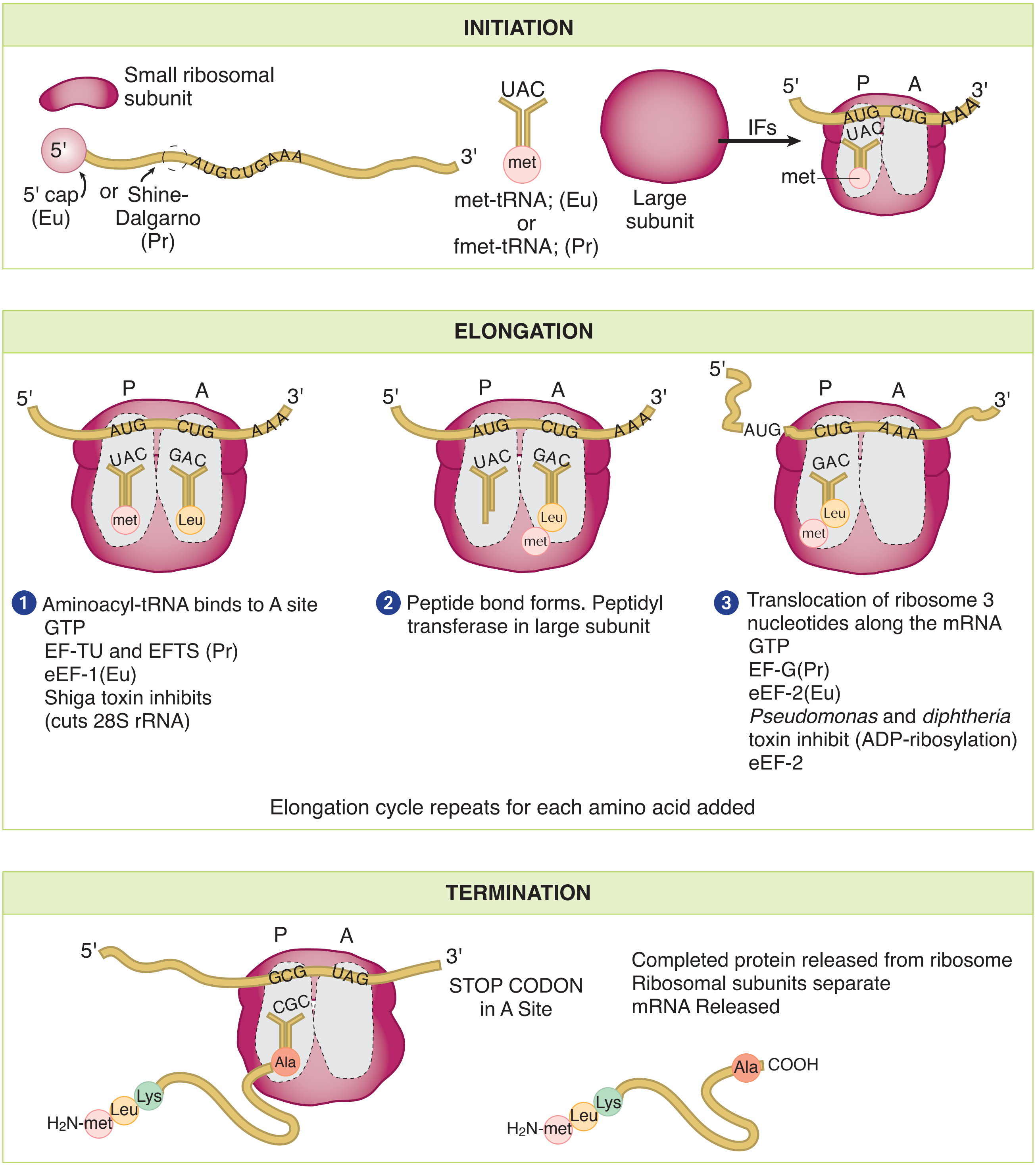


Figure I-4-6. Steps in Translation

Initiation

The small ribosomal subunit binds to the mRNA. In prokaryotes, the 16S rRNA of the small subunit binds to the Shine-Dalgarno sequence in the 5′ untranslated region of the mRNA. In eukaryotes, the small subunit binds to the 5′ cap structure and slides down the message to the first AUG.

The charged initiator tRNA becomes bound to the AUG start codon on the message through base pairing with its anticodon. The initiator tRNA in prokaryotes carries fMet, whereas the initiator tRNA in eukaryotes carries Met.

The large subunit binds to the small subunit, forming the completed initiation complex.

There are 2 important binding sites on the ribosome called the P site and the A site.

- The peptidyl site (P site) is the site on the ribosome where fMet–$tRNA_i$ initially binds. After formation of the first peptide bond, the P site is a binding site for the growing peptide chain.
- The aminoacyl site (A site) binds each new incoming tRNA molecule carrying an activated amino acid.

Elongation

Elongation is a 3-step cycle that is repeated for each amino acid added to the protein after the initiator methionine. Each cycle uses 4 high-energy bonds (2 from the ATP used in amino acid activation to charge the tRNA, and 2 from GTP). During elongation, the ribosome moves in the 5′ to 3′ direction along the mRNA, synthesizing the protein from amino to carboxyl terminus. The 3 steps are:

- A charged tRNA binds in the A site. The particular aminoacyl–tRNA is determined by the mRNA codon aligned with the A site.
- Peptidyl transferase, an enzyme that is part of the large subunit, forms the peptide bond between the new amino acid and the carboxyl end of the growing polypeptide chain. The bond linking the growing peptide to the tRNA in the P site is broken, and the growing peptide attaches to the tRNA located in the A site.
- In the translocation step, the ribosome moves exactly 3 nucleotides (one codon) along the message. This moves the growing peptidyl–tRNA into the P site and aligns the next codon to be translated with the empty A site.

In eukaryotic cells, elongation factor-2 (eEF-2) used in translocation is inactivated through ADP-ribosylation by *Pseudomonas* and *Diphtheria* toxins.

Shiga and Shiga-like toxins clip an adenine residue from the 28S rRNA in the 60S subunit stopping protein synthesis in eukaryotic cells.

Termination

When any of the 3 stop (termination or nonsense) codons moves into the A site, peptidyl transferase (with the help of release factor) hydrolyzes the completed protein from the final tRNA in the P site. The mRNA, ribosome, tRNA, and factors can all be reused for additional protein synthesis.

Clinical Correlate

Gray baby syndrome is a dangerous condition that occurs in newborns (especially premature babies) who are given the drug chloramphenicol. Chloramphenicol is a drug used to fight bacterial infections, including meningitis. If given to a newborn, however, this drug can trigger a potentially deadly reaction. Babies do not have sufficient UDP-glucuronyl transferase activity needed to allow excretion of this drug. The drug builds up in the baby's bloodstream and can lead to:

- Blue lips, nail beds, and skin (cyanosis)
- Death
- Low blood pressure

Recall Question

Which of the following terms is used to describe a disease that, from generation to generation, shows a decrease in the age of onset and an increase in the severity of symptoms?

A. Acception

B. Assumption

C. Anticipation

D. Mutation

Answer: C

POLYSOMES

Messenger RNA molecules are very long compared with the size of a ribosome, allowing room for several ribosomes to translate a message at the same time. Because ribosomes translate mRNA in the 5′ to 3′ direction, the ribosome closest to the 3′ end has the longest nascent peptide. Polysomes are found free in the cytoplasm or attached to the rough endoplasmic reticulum (RER), depending on the protein being translated.

INHIBITORS OF PROTEIN SYNTHESIS

Some well-known inhibitors of prokaryotic translation include streptomycin, erythromycin, tetracycline, and chloramphenicol. Inhibitors of eukaryotic translation include cycloheximide and *Diphtheria* and *Pseudomonas* toxins.

Certain antibiotics (for example, chloramphenicol) inhibit mitochondrial protein synthesis, but not cytoplasmic protein synthesis, because mitochondrial ribosomes are similar to prokaryotic ribosomes.

PROTEIN FOLDING AND SUBUNIT ASSEMBLY

As proteins emerge from ribosomes, they fold into 3-dimensional conformations that are essential for their subsequent biologic activity. Generally, 4 levels of protein shape are distinguished:

Primary—sequence of amino acids specified in the gene.

Secondary—folding of the amino acid chain into an energetically stable structure. Two common examples are the α-helix and the β-pleated sheet. These shapes are reinforced by hydrogen bonds. An individual protein may contain both types of secondary structures. Some proteins, like collagen, contain neither but have their own more characteristic secondary structures.

Tertiary—positioning of the secondary structures in relation to each other to generate higher-order 3-dimensional shapes (the domains of the IgG molecule are examples). Tertiary structure also includes the shape of the protein as a whole (globular, fibrous). Tertiary structures are stabilized by weak bonds (hydrogen,hydrophobic, ionic) and, in some proteins, strong, covalent disulfide bonds. Agents such as heat or urea disrupt tertiary structure to denature proteins, causing loss of function.

Quaternary—in proteins such as hemoglobin that have multiple subunits, quaternary structure describes the interactions among subunits.

TRANSLATION OCCURS ON FREE RIBOSOMES AND ON THE ROUGH ENDOPLASMIC RETICULUM

Although all translation of eukaryotic nuclear genes begins on ribosomes free in the cytoplasm, the proteins being translated may belong in other locations. For example, certain proteins are translated on ribosomes associated with the rough endoplasmic reticulum (RER), including:

- Secreted proteins
- Proteins inserted into the cell membrane
- Lysosomal enzymes

Proteins translated on free cytoplasmic ribosomes include:

- Cytoplasmic proteins
- Mitochondrial proteins (encoded by nuclear genes)

Molecular Chaperones and Proteasomes

High-Yield

Protein folding is an essential step in the final synthesis of any protein. There is a class of specialized proteins, **chaperones**, whose function is to assist in this process. Molecular chaperones function in many cell compartments, including the endoplasmic reticulum, where extensive protein synthesis occurs. Failure to fold correctly usually results in eventual destruction of the protein.

Proteasomes and Ubiquitin

High-Yield

Whenever protein synthesis occurs in a cell, a few copies of a particular protein may not fold correctly. These defective copies are covalently marked for destruction by the addition of multiple copies of ubiquitin. Polyubiquinated proteins are directed to proteasomes for destruction. Proteasomes are large, cytoplasmic complexes that have multiple protease activities capable of digesting damaged proteins to peptides. Proteasomes also play a role in producing antigenic peptides for presentation by class I MHC molecules.

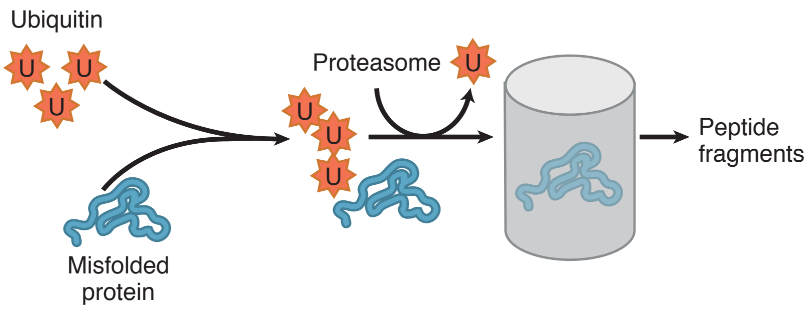


Figure I-4-7. Degradation of Misfolded Proteins by Proteasomes

Many proteins require signals to ensure delivery to the appropriate organelles. Especially important among these signals are:

- The *N*-terminal hydrophobic signal sequence used to ensure translation on the RER
- Phosphorylation of mannose residues important for directing an enzyme to a lysosome

The targeting process for these proteins is illustrated below.

Clinical Correlate

Cystic Fibrosis

The majority of cases of cystic fibrosis result from the deletion of phenylalanine at position 508 (ΔF508), which interferes with proper protein folding and the posttranslational processing of oligosaccharide side chains. The abnormal chloride channel protein (CFTR) is degraded by the cytosolic proteasome complex rather than being translocated to the cell membrane. Other functional defects in CFTR protein reaching the cell membrane may also contribute to the pathogenesis of cystic fibrosis.

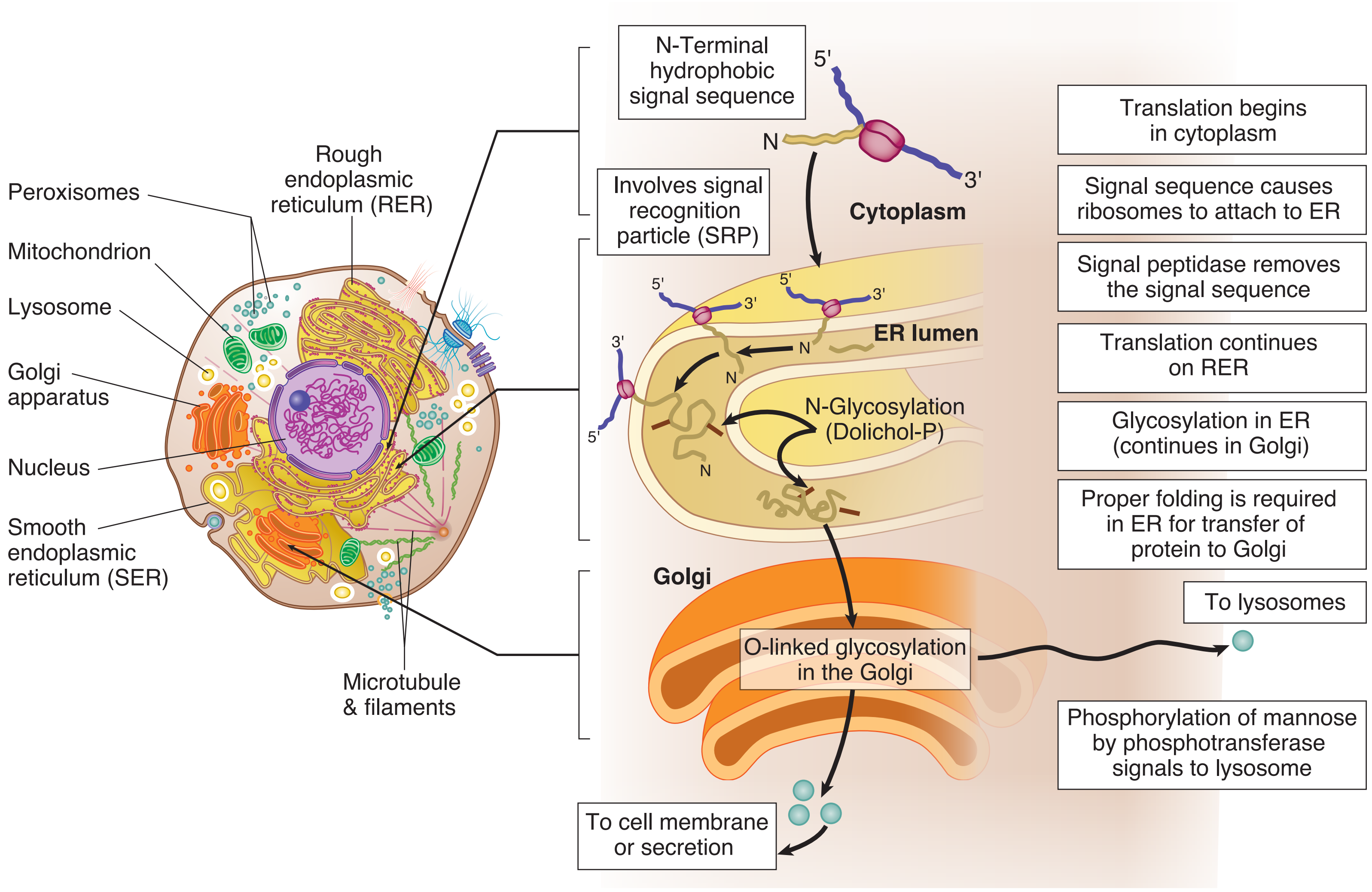


Figure I-4-8. Synthesis of Secretory, Membrane, and Lysosomal Proteins

N-Terminal Hydrophobic Signal Sequence

High-Yield

This sequence is found on proteins destined to be secreted (insulin), placed in the cell membrane (Na^+-K^+ ATPase), or ultimately directed to the lysosome (sphingomyelinase). These proteins all require N-terminal hydrophobic signal sequences as part of their primary structure. Translation begins on free cytoplasmic ribosomes, but after translation of the signal sequence, the ribosome is positioned on the ER (now RER) with the help of a signal recognition particle. During translation, the nascent protein is fed through the membrane of the RER and captured in the lumen. The signal sequence is cleaved off in the ER, and then the protein passes into the Golgi for further modification and sorting.

In transit through the ER and Golgi, most proteins acquire oligosaccharide side chains, becoming glycoproteins. N-glycosylation refers to the addition of sugar chains to the nitrogen of asparagine residues (N-linked). The attachment of sugars in N-glycosylation begins in the ER (cotranslational modification) and requires the participation of a special lipid called dolichol phosphate. The N-linked sugar chain can further be modified upon entry in the Golgi (posttranslational modification).

O-glycosylation refers to the addition of sugar chains to the hydroxyl group of either serine or threonine residues of the protein, and it occurs exclusively in the Golgi (posttranslational modification). Depending of the particular glycoprotein, some proteins are solely N-glycosylated (for example, transferrin); some are solely O-glycosylated (for example, heparin); and some are both N- and O-glycosylated (for example, LDL receptor). Significantly, the structure and sequence of the oligosaccharide chains on proteins and lipids (glycolipids) are the basis of the A, B, O blood groups.

Accumulation or ineffective targeting of misfolded proteins

Proteins synthesized in the endoplasmic reticulum must fold correctly for transport to the Golgi and then to their final destinations. In certain genetic diseases, the mutation may cause all copies of the protein to fold incorrectly. The result is loss of protein function and, in some cases, accumulation of the misfolded protein in the endoplasmic reticulum.

α_1-Antitrypsin Deficiency

A 70-year-old woman with elevated liver function tests was being evaluated for cirrhosis. Her serum α_1-antitrypsin level was 25 mg/dL (normal 90–225 mg/dL). A liver biopsy showed micronodular cirrhosis and prominent fibrosis. Immunohistochemical studies showed intense staining with α_1-antitrypsin antibody. The patient was tested for likely mutations in the α_1-antitrypsin gene and found to be homozygous for the Z mutation (ZZ). This mutation causes the α_1-antitrypsin protein to misfold and aggregate in the endoplastic reticulum, where it damages cells, eventually leading to cirrhosis. She had no evidence of pulmonary disease.

α_1-antitrypsin is a protein synthesized primarily by the liver and secreted in the bloodstream. Its function is to protect cells by serving as an inhibitor of proteases released during a normal inflammatory response. Among the more than 90 allelic variants of the α_1-antitrypsin gene, the Z and S variants are most often encountered with α_1-antitrypsin deficiency. Both are the result of point mutations, which can be detected with the polymerase chain reaction (PCR) technique.

Lysosomal Enzymes and Phosphorylation of Mannose

High-Yield

Lysosomal enzymes are glycosylated and modified in a characteristic way. Most importantly, when they arrive in the Golgi apparatus, specific mannose residues located in their N-linked oligosaccharide chains are phosphorylated by N-acetylglucosamine-1 phosphotransferase, forming a critical mannose-6-phosphate in the oligosaccharide chain. This phosphorylation is the critical event that removes them from the secretion pathway and directs them to lysosomes. Genetic defects affecting this phosphorylation produce I-cell disease in which lysosomal enzymes are released into the extracellular space, and inclusion bodies accumulate in the cell, compromising its function.

Major Symptoms of I-Cell Disease

A child aged 5 months was referred to a specialist. The child had been born with dislocated hips and a coarse featured face. He had been suffering repeated upper respiratory tract infections and did not seem to be developing his motor abilities, Clinical examination revealed hyperplasia of the gums, restriction of joint mobility and hepatosplenomegaly. On listening to the heart a mitral valve murmur could be detected. Further investigation involved cell culture of the child's fibroblasts obtained from a skin biopsy. Examination of the fibroblasts under the microscope revealed the presence of numerous intracellular inclusions, which on electron microscopy were revealed to be large lysosomes. Biochemical analysis showed decreased levels of the lysosomal hydrolase ß-glucuronidase within the fibroblasts, but elevated levels of this enzyme within the culture medium. A diagnosis of I-cell disease was made.

- Coarse facial features, gingival hyperplasia, macroglossia
- Craniofacial abnormalities, joint immobility, clubfoot, claw-hand, scoliosis
- Psychomotor retardation, growth retardation
- Cardiorespiratory failure, death in first decade
- Bone fracture and deformities
- Mitral valve defect
- Secretion of active lysosomal enzymes into blood and extracellular fluid

CO- AND POSTTRANSLATIONAL COVALENT MODIFICATIONS

In addition to disulfide bond formation while proteins are folding, other covalent modifications include:

- Glycosylation: addition of oligosaccharide as proteins pass through the ER and Golgi apparatus
- Proteolysis: cleavage of peptide bonds to remodel proteins and activate them (proinsulin, trypsinogen, prothrombin)
- Phosphorylation: addition of phosphate by protein kinases

Bridge to Anatomy

Lysosomes

- Organelles whose major function is to digest materials that the cell has ingested by endocytosis.
- Contain multiple enzymes that, collectively, digest carbohydrates (glycosylases), lipids (lipases), and proteins (proteases).
- Especially prominent in cells such as neutrophils and macrophages, though they serve this essential role in almost all cells.

When a lysosomal enzyme is missing (for instance in a genetic disease such as Tay-Sachs), the undigested substrate accumulates in the cell, often leading to serious consequences.

- γ-Carboxylation: produces Ca^{2+} binding sites
- Prenylation: addition of farnesyl or geranylgeranyl lipid groups to certain membrane-associated proteins

POSTTRANSLATIONAL MODIFICATIONS OF COLLAGEN

Collagen is an example of a protein that undergoes several important co- and posttranslational modifications. It has a somewhat unique primary structure in that much of its length is composed of a repeating tripeptide Gly-X-Y-Gly-X-Y-etc. Hydroxyproline is an amino acid unique to collagen. The hydroxyproline is produced by hydroxylation of prolyl residues at the Y positions in procollagen chains as they pass through the RER.

1. Prepro-α chains containing a hydrophobic signal sequence are synthesized by ribosomes attached to the RER.
2. The hydrophobic signal sequence is removed by signal peptidase in the RER to form pro-α chains.
3. Selected prolines and lysines are hydroxylated by prolyl and lysyl hydroxylases. These enzymes, located in the RER, require ascorbate (vitamin C), deficiency of which produces scurvy.
4. Selected hydroxylysines are glycosylated.
5. Three pro-α chains assemble to form a triple helical structure (procollagen), which can now be transferred to the Golgi. Modification of oligosaccharide continues in the Golgi.
6. Procollagen is secreted from the cell.
7. The propeptides are cleaved from the ends of procollagen by proteases to form collagen molecules (also called tropocollagen).
8. Collagen molecules assemble into fibrils. Cross-linking involves lysyl oxidase, an enzyme that requires O_2 and copper.
9. Fibrils aggregate and cross-link to form collagen fibers.

Table I-4-3. Collagen

Collagen Type	Characteristics	Tissue Distribution	Associated Diseases
I	Bundles of fibers High tensile strength	Bone, skin, tendons	Osteogenesis imperfecta Ehlers-Danlos (various)
II	Thin fibrils Structural	Cartilage Vitreous humor	----------
III	Thin fibrils Pliable	Blood vessels Granulation tissue	Ehlers-Danlos Type IV Keloid formation
IV	Amorphous	Basement membranes	Goodpasture syndrome Alport disease

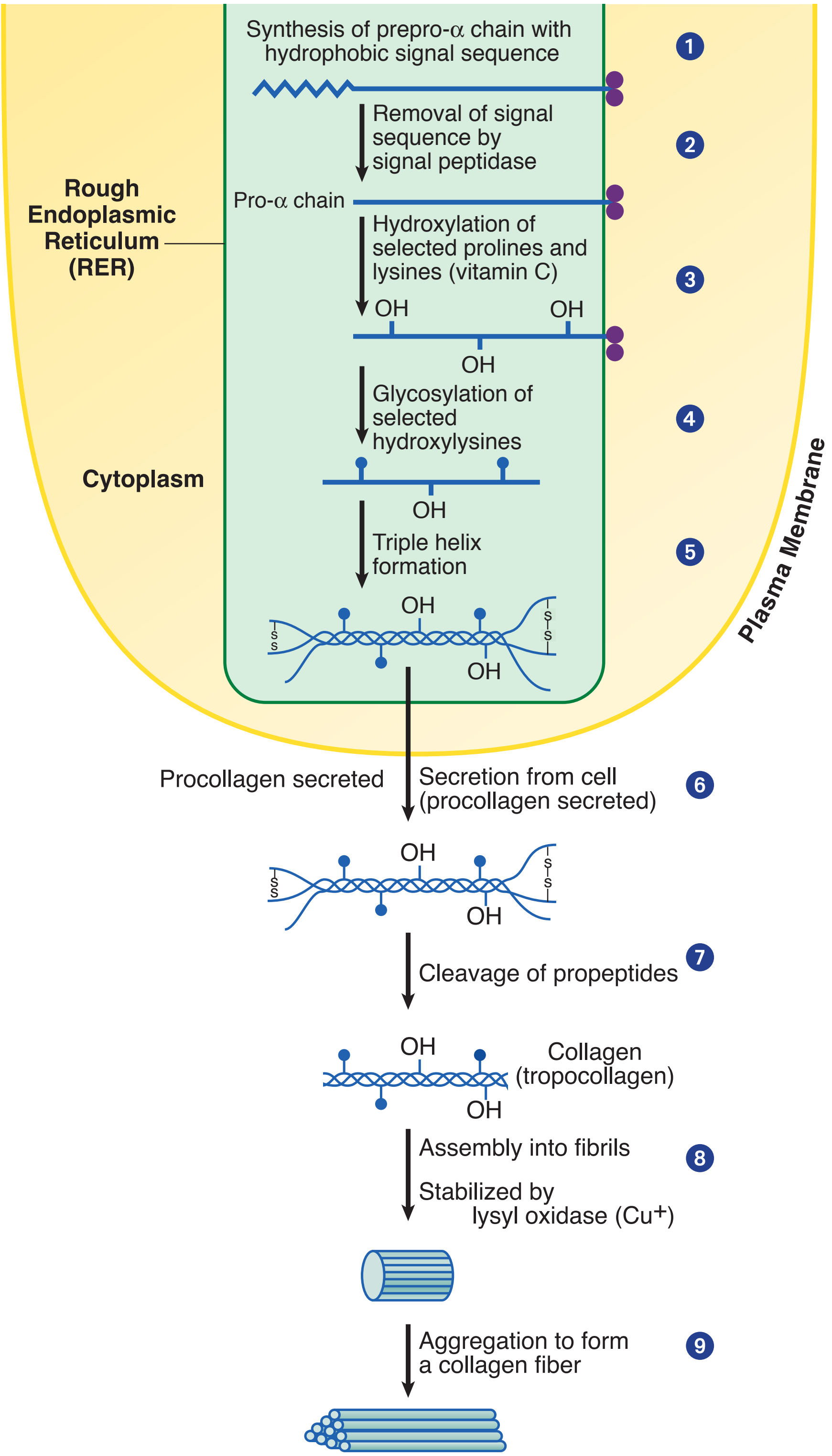


Figure I-4-9. Synthesis of Collagen

Clinical Correlate

Ehlers-Danlos (ED) syndromes represent a collection of defects in the normal synthesis and processing of collagen. Like osteogenesis imperfecta, these syndromes are a result of locus heterogeneity in which defects in several different genes (loci) can result in similar symptoms.

ED Type IV, the vascular type, is an autosomal dominant disease caused by mutations in the gene for type-3 procollagen. Characteristic features include thin, translucent skin; arterial, intestinal, or uterine rupture; and easy bruising.

Also see Section II, Chapter 1; Locus Heterogeneity.

There are several important diseases associated with defective collagen production.

Table I-4-4. Disorders of Collagen Biosynthesis

Disease	Defect	Major Symptoms
Scurvy	Deficient hydroxylation secondary to ascorbate deficiency	Petechiae, ecchymoses Loose teeth, bleeding gums Poor wound healing Poor bone development
Osteogenesis imperfecta	Mutations in collagen genes	Skeletal deformities Fractures, blue sclera
Ehlers-Danlos syndromes	Mutations in collagen genes and proline and lysyl hydroxylases	Hyperextensible, fragile skin Hypermobile joints, dislocations, varicose veins, ecchymoses, arterial, intestinal ruptures
Menkes disease	Deficient cross-linking secondary to functional copper deficiency	Depigmented (steely) hair Arterial tortuosity, rupture Cerebral degeneration Osteoporosis, anemia

Recall Question

Vitreous humor is formed from which type of collagen?

A. Type I

B. Type II

C. Type III

D. Type IV

Answer: B

Menkes Disease

> A 4-month-old infant who failed to grow and appeared to be mentally retarded was brought to the clinic for testing. The physician noted that the infant had abnormally kinky and hypopigmented hair. An arteriogram showed elongation and tortuosity of the major arteries. Additional tests revealed bladder diverticula and subdural hematomas. A blood test showed that the infant had low serum ceruloplasmin and only 10% of normal serum copper levels.

This infant has Menkes disease, which is also known as Ehlers-Danlos syndrome type IX (kinky hair syndrome). It is an X-linked recessive disease that has an incidence of 1/100,000 newborns. Common with Ehlers-Danlos diseases, Menkes disease has a symptomology due, in part, to weak collagen.

The disease is caused by mutations in the gene ATP7A, which encodes an ATP-dependent copper efflux protein in the intestine. Copper can be absorbed into the mucosal cell, but it cannot be transported into the bloodstream. Consequently, an affected individual will have severe copper deficiency and all copper-requiring enzymes will be adversely affected. Lysyl oxidase requires copper and plays a direct role in collagen formation by catalyzing the cross-linking of collagen fibrils. A deficiency in the activity of this enzyme and other copper-dependent enzymes would be directly responsible for the described symptoms in this infant.

Table I-4-5. Important Points About the Genetic Code, Mutations, and Translation

	Prokaryotic	Eukaryotic
Genetic code	Start: AUG (also codes for Met) Stop: UAG, UGA, UAA Unambiguous (1 codon = 1 amino acid) Redundant (1 amino acid >1 codon); often differ at base 3	
Mutations	Point mutations: silent, missense, nonsense Frameshift (delete 1 or 2 nucleotides; not multiple of 3) Large segment deletion	
		Mutation in splice site Trinucleotide repeat expansion
Amino acid activation	Aminoacyl-tRNA synthetase: two high-energy bonds (ATP) to link amino acid to tRNA	
Translation: Initiation	30S subunit binds to Shine-Dalgarno sequence on mRNA fMet–$tRNA_i$ binds to P site GTP required	40S subunit associates with 5′ cap on mRNA Met–$tRNA_i$ binds to P site GTP required
Translation: Elongation	Charged aminoacyl–tRNA binds to A site (GTP) Peptide bond forms (two high-energy bonds from amino acid activation) Peptidyl transferase (50S subunit) Translocation: GTP required	Charged aminoacyl–tRNA binds to A site (GTP) 28S rRNA is cut by Shiga and Shiga-like toxins removing an adenine residue. Prevents protein synthesis. Peptide bond forms (two high-energy bonds from amino acid activation) Peptidyl transferase (60S subunit) Translocation: GTP required eEF-2 inhibited by *Diphtheria* and *Pseudomonas* toxins
Termination	Release of protein; protein synthesized N to C	
Protein targeting		Secreted or membrane proteins: N-terminal hydrophobic signal sequence Lysosomal enzymes: phosphorylation of mannose by phosphotransferase in Golgi I-cell disease
Other important disease associations		Scurvy (prolyl hydroxylase, Vit C) Menke Disease (Cu deficiency, lysyl oxidase)

Review Questions

Select the ONE best answer.

1. In the genetic code of human nuclear DNA, one of the codons specifying the amino acid tyrosine is UAC. Another codon specifying this same amino acid is

   A. AAC

   B. UAG

   C. UCC

   D. AUG

   E. UAU

Items 2 and 3

A. ATGCAA...→ ATG**T**AA

B. ATGAAA...→ **G**TGAAA

C. TATAAG...→ T**C**TAAG

D. CTTAAG...→ **G**TTAAG

E. ATGAAT...→ ATG**C**AT

The options above represent mutations in the DNA with base changes indicated in boldface type. For each mutation described in the questions below, choose the most closely related sequence change in the options above.

2. Nonsense mutation

3. Mutation decreasing the initiation of transcription

4. Accumulation of heme in reticulocytes can regulate globin synthesis by indirectly inactivating eIF-2. Which of the following steps is most directly affected by this mechanism?

   A. Attachment of spliceosomes to pre-mRNA

   B. Attachment of the ribosome to the endoplasmic reticulum

   C. Met-tRNAmet binding to the P-site

   D. Translocation of mRNA on the ribosome

   E. Attachment of RNA polymerase II to the promoter

5. A nasopharyngeal swab obtained from a 4-month-old infant with rhinitis and paroxysmal coughing tested positive upon culture for *Bordetella pertussis*. He was admitted to the hospital for therapy with an antibiotic that inhibits the translocation of the 70S ribosomes on the mRNA. This patient was most likely treated with

   A. erythromycin

   B. tetracycline

   C. chloramphenicol

   D. rifamycin

   E. levofloxacin

6. A 25-month-old Caucasian girl has coarse facial features and gingival hyperplasia and, at 2 months of age, began developing multiple, progressive symptoms of mental retardation, joint contractures, hepatomegaly, and cardiomegaly. Levels of lysosomal enzymes are elevated in her serum, and fibroblasts show phase-dense inclusions in the cytoplasm. Which of the following enzyme deficiencies is most consistent with these observations?

   A. Golgi-associated phosphotransferase
   B. Lysosomal α-1,4-glucosidase
   C. Endoplasmic reticulum–associated signal peptidase
   D. Cytoplasmic α-1,4-phosphorylase
   E. Lysosomal hexosaminidase A

7. Parahemophilia is an autosomal recessive bleeding disorder characterized by a reduced plasma concentration of the Factor V blood coagulation protein. Deficiency arises from a 12 base-pair deletion in the Factor V gene that impairs the secretion of Factor V by hepatocytes and results in an abnormal accumulation of immunoreactive Factor V antigen in the cytoplasm. In which region of the Factor V gene would this mutation most likely be located?

   A. 5′ Untranslated region
   B. First exon
   C. Middle intron
   D. Last exon
   E. 3′ Untranslated region

8. Collagen, the most abundant protein in the human body, is present in varying amounts in many tissues. If one wished to compare the collagen content of several tissues, one could measure their content of

   A. glycine
   B. proline
   C. hydroxyproline
   D. cysteine
   E. lysine

9. A 6-month-old infant is seen in the emergency room with a fractured rib and subdural hematoma. The child's hair is thin, colorless, and tangled. His serum copper level is 5.5 nM (normal for age, 11–12 nM). Developmental delay is prominent. A deficiency of which enzyme activity most closely relates to these symptoms?

   A. Lysyl oxidase
   B. Prolyl hydroxylase
   C. γ-Glutamyl carboxylase
   D. Phosphotransferase in Golgi
   E. α-1,4-glucosidase

10. Respiratory tract infections caused by *Pseudomonas aeruginosa* are associated with the secretion of exotoxin A by this organism. What effect will this toxin most likely have on eukaryotic cells?

    A. Stimulation of nitric oxide (NO) synthesis
    B. ADP-ribosylation of a Gs protein
    C. ADP-ribosylation of eEF-2
    D. ADP-ribosylation of a Gi protein
    E. Stimulation of histamine release

11. A 4-year-old toddler with cystic fibrosis (CF) is seen by his physician for an upper respiratory infection with *Pseudomonas aeruginosa*. He is started on oral ciprofloxacin and is referred to a CF center as a potential candidate for gene therapy. Prior genetic testing of the patient identified the mutation causing CF as a 3-base-pair deletion in exon 10 of the CF gene. The nucleotide sequences of codons 506–511 in this region of the normal and mutant alleles are compared below.

Codon Number	506	507	508	509	510	511
Normal Gene	ATC	ATC	TTT	GGT	GTT	TCC
Mutant Gene	ATC	AT•	••T	GGT	GTT	TCC

3-base deletion

What effect will this patient's mutation have on the amino acid sequence of the protein encoded by the CF gene?

	U	C	A	G	
U	UUU UUC } Phe UUA UUG } Leu	UCU UCC UCA UCG } Ser	UAU UAC } Tyr UAA UAG } Stop	UGU UGC } Cys UGA Stop UGG Trp	U C A G
C	CUU CUC CUA CUG } Leu	CCU CCC CCA CCG } Pro	CAU CAC } His CAA CAG } Gln	CGU CGC CGA CGG } Arg	U C A G
A	AUU AUC AUA } Ile AUG Met	ACU ACC ACA ACG } Thr	AAU AAC } Asn AAA AAG } Lys	AGU AGC } Ser AGA AGG } Arg	U C A G
G	GUU GUC GUA GUG } Val	GCU GCC GCA GCG } Ala	GAU GAC } Asp GAA GAG } Glu	GGU GGC GGA GGG } Gly	U C A G

A. Deletion of a phenylalanine residue with no change in C-terminal sequence

B. Deletion of a leucine residue causing a change in the C-terminal sequence

C. Deletion of a phenylalanine residue causing a change in the C-terminal sequence

D. Deletion of a leucine residue with no change in C-terminal sequence

12. A 10-year-old boy with severe progressive skin ulceration, decreased resistance to infection, and impaired cognitive ability has been diagnosed with a genetic deficiency of the enzyme prolidase. Mutation analysis has identified a single base substitution at the 3' end of intron 6 of the mutant allele as well as deletion of a 45-base exon (exon 7) in the prolidase cDNA. Which type of gene mutation was most likely inherited by this boy?

A. Frameshift mutation

B. In-frame mutation

C. Missense mutation

D. Nonsense mutation

E. Splice site mutation

Answers

1. **Answer: E.** Because of wobble codons for the same amino acid often differ in the third base. Option B would be acceptable, except that it is a stop codon.

2. **Answer: A.** The sequence now contains TAA which will be transcribed to UAA in the mRNA.

3. **Answer: C.** The transcription promoter TATA has been changed to TCTA. Don't choose the distractor B. The question is not about translation.

4. **Answer: C.** eIF-2 designates a protein factor of the initiation phase in eukaryotic translation. The only event listed that would occur during this phase is placement of initiator tRNA in the P-site.

5. **Answer: A.** Erythromycin is the antibiotic of choice for pertussis. It inhibits translocation.

6. **Answer: A.** Characteristic symptoms of I-cell disease. Note release of lysosomal enzymes into serum, which would not be seen in the other deficiencies.

7. **Answer: B.** Decreased factor V secretion and a corresponding accumulation of cytoplasmic antigen suggest a defect in the translocation of the nascent protein to the endoplasmic reticulum. This implies a mutation in the N-terminal amino acid signal sequence required for targeting to the ER and encoded by the first exon of the gene.

8. **Answer: C.** Hydroxyproline is found uniquely in collagen. Although collagen is also rich in glycine, many other proteins contain significant amounts of glycine.

9. **Answer: A.** The child has Menkes disease, in which cellular copper transport is abnormal and produces a functional copper deficiency. Lysyl oxidase in collagen metabolism requires copper. His fragile bones and blood vessels result from weak, poorly crosslinked connective tissue.

10. **Answer: C.** *Pseudomonas* and diphtheria toxins inhibit eEF-2, the translocation factor in eukaryotic translation.

11. **Answer: A.** Deletion of CTT results only in the loss of phe 508; ile 507 and the C-terminal sequence are unaltered because ATC and ATT both code for ile (the coding sequence is unchanged).

12. **Answer: E.** A base *substitution* at an intron-exon junction, which leads to the deletion of an entire exon is indicative of a splice site mutation.

Regulation of Eukaryotic Gene Expression

5

Learning Objectives

- ❑ Demonstrate understanding of regulation of eukaryotic gene expression
- ❑ Know concept and examples of specific transcription factors

GENETIC REGULATION

Regulation of gene expression is an essential feature in maintaining the functional integrity of a cell. Increasing or decreasing the expression of a gene can occur through various mechanisms, but many of the important ones involve regulating the rate of transcription. In addition to the basic transcription proteins, RNA polymerase and TFIID in eukaryotes activator and repressor proteins help control the rate of the process. These regulatory proteins bind to specific DNA sequences (enhancer or silencer elements) associated with eukaryotic gene regions.

Other mechanisms are important, and gene expression is controlled at multiple levels.

REGULATION OF EUKARYOTIC GENE EXPRESSION

In eukaryotic cells, DNA is packaged in chromatin structures, and gene expression typically requires chromatin remodeling in order to make the desired gene region accessible to RNA polymerase and other proteins (transcription factors) required for gene expression. Important aspects of chromatin remodeling include:

- Transcription factors that bind to the DNA and recruit other coactivators such as histone acetylases
- Histone acetylases (favor gene expression) and deacetylases (favor inactive chromatin)
- Certain lysyl residues in the histones are acetylated decreasing the positive charge and weakening the interaction with DNA.
- A chromatin remodeling engine that binds to acetylated lysyl residues and reconfigures the DNA to expose the promoter region.
- Additional transcription factors bind in the promoter region and recruit RNA polymerase.

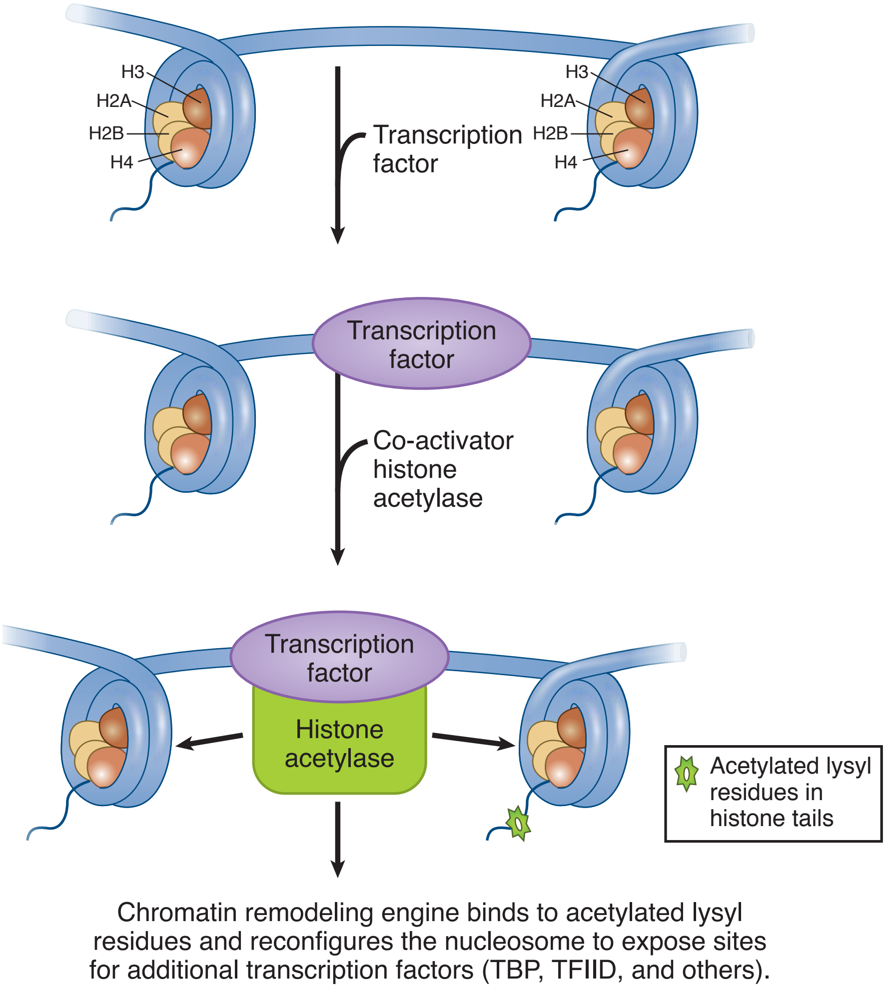


Figure I-5-1. Chromatin Remodeling

Once the transcription complex is formed, basal (low level) transcription occurs, maintaining moderate but adequate levels of the protein encoded by this gene in the cell. The transcription factors assembled in this complex are referred to as general transcription factors.

There are times when the expression of the gene should be increased in response to specific signals such as hormones, growth factors, intracellular conditions. In this case there are DNA sequences referred to as response elements that bind specific transcription factors. Several of these response elements may be grouped together to form an enhancer that allows control of gene expression by multiple signals.

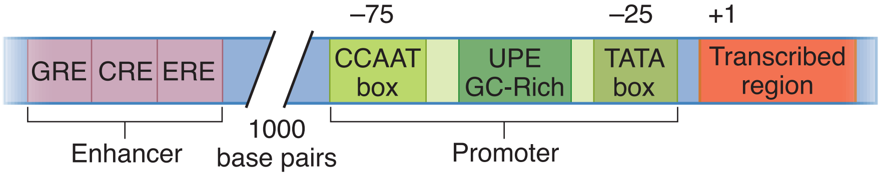


Figure I-5-2. Enhancers and Upstream Promoter Elements

Upstream Promoter Elements

Only the proximity of the upstream promoter element to the –25 sequence distinguishes it from an enhancer. Upstream promoter elements include:

- CCAAT box (around –75) that binds a transcription factor NF-1
- GC-rich sequence that binds a general transcription factor SP-1

Enhancers

High-Yield

Enhancers in the DNA are binding sites for activator proteins. Enhancers have the following characteristics:

- They may be up to 1,000 base pairs away from the gene.
- They may be located upstream, downstream, or within an intron of the gene they control.
- The orientation of the enhancer sequence with respect to the gene is not important.
- Enhancers can appear to act in a tissue-specific manner if the DNA-binding proteins that interact with them are present only in certain tissues.
- Enhancers may be brought close to the basal promoter region in space by bending of the DNA molecule.

Similar sequences that bind repressor proteins in eukaryotes are called silencers. There are fewer examples of these sequences known.

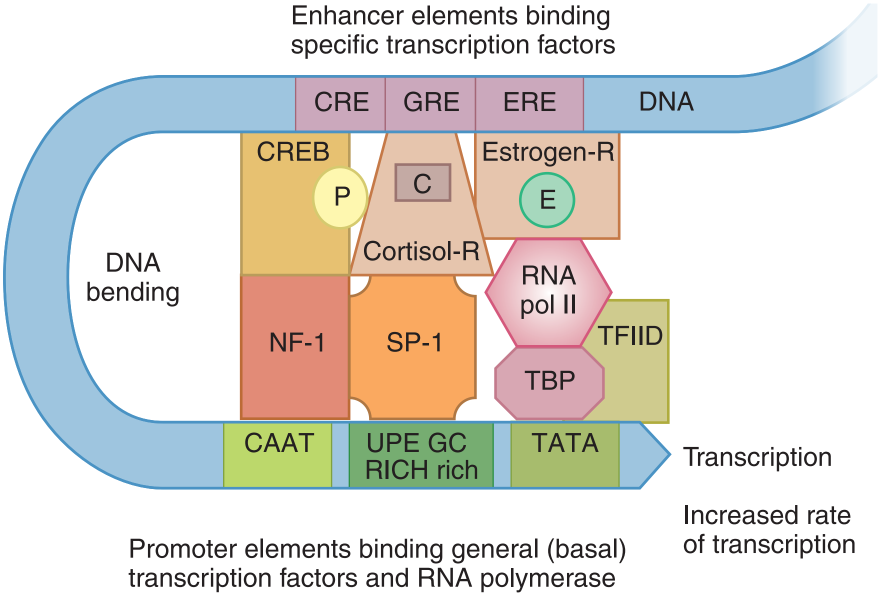


Figure I-5-3. Stimulation of Transcription by an Enhancer and Its Associated Transcription Factors

Note

The Ig heavy chain locus has an enhancer in the large intron separating the coding regions for the variable domain from the coding regions for the constant domains.

Note

The DNA regulatory base sequences (e.g., promoters, enhancers, response elements, and UPEs) in the vicinity of genes that serve as binding sites for proteins are often called **"*cis*" regulators**.

Transcription factors (and the genes that code for them) are called **"*trans*" regulators**. *Trans* regulatory proteins can diffuse through the cell to their point of action.

Transcription Factors

High-Yield

The activator proteins that bind response elements are often referred to as transcription factors. Typically, transcription factors contain at least 2 recognizable domains, a DNA-binding domain and an activation domain.

- The **DNA-binding domain** binds to a specific nucleotide sequence in the promoter or response element. Several types of DNA-binding domain motifs have been characterized and have been used to define certain families of transcription factors. Some common DNA-binding domains include:
  - Zinc fingers (steroid hormone receptors)
  - Leucine zippers (cAMP-dependent transcription factor)
  - Helix-loop-helix
  - Helix-turn-helix (homeodomain proteins encoded by homeotic/homeobox genes)
- The **activation domain** allows the transcription factor to:
  - Bind to other transcription factors and coregulators
  - Interact with RNA polymerase II to stabilize the formation of the initiation complex
  - Recruit chromatin-modifying proteins such as histone acetylases or deacetylases

Two types can be distinguished: general transcription factors and specific transcription factors.

Table I-5-1. Important Specific Transcription Factors

Transcription Factor (DNA-Binding Protein)	Response Element (Binding Site)	Function	Protein Class
Steroid receptors	HRE	Steroid response	Zinc finger
cAMP response element binding (CREB) protein	CRE	Response to cAMP	Leucine zipper
Peroxisome proliferator-activated receptors (PPARs)	PPREs	Regulate multiple aspects of lipid metabolism Activated by fibrates and thiazolidinediones	Zinc finger
NFkB (nuclear factor kappa-B)	kB elements	Regulates expression of many genes in immune system	Rel domains
Homeodomain proteins	——	Regulate gene expression during development	Helix-turn-helix

General Transcription Factors

In eukaryotes, general transcription factors must bind to the promoter to allow RNA polymerase II to bind and form the initiation complex at the start site for transcription. General transcription factors are common to most genes. The general

transcription factor TFIID with its TATA box-binding protein subunit (TBP) must bind to the TATA box before RNA polymerase II can bind. Other examples include SP-1 and NF-1 that modulate basal transcription of many genes.

Specific Transcription Factors

High-Yield

Specific transcription factors bind to enhancer regions or, in a few cases, to silencers and modulate the formation of the initiation complex, thus regulating the rate of initiation of transcription. Each gene contains a variety of enhancer or silencer sequences in its regulatory region. The exact combination of specific transcription factors available (and active) in a particular cell at a particular time determines which genes will be transcribed at what rates. Because specific transcription factors are proteins, their expression can be cell-type specific. Additionally, hormones may regulate the activity of some specific transcription factors. Examples include steroid receptors and the CREB protein.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that bind to DNA response elements (PPREs) and control multiple aspects of lipid metabolism. Individual members of this family of zinc-finger proteins are activated by a variety of natural and xenobiotic ligands, including:

- Fatty acids
- Prostaglandin derivatives
- Fibrates
- Thiazolidinediones

The improvement in insulin resistance seen with thiazolidinediones is thought to be mediated through their interaction with PPARγ. Clofibrate binds PPARα, affecting different aspects of lipid metabolism than the thiazolidinediones.

Peroxisomes: Hypertriglyceridemia and Fibrates

A 50-year-old man sees his physician for increasingly frequent episodes of acute pain in his upper abdomen and back after meals. The physician orders fasting blood tests and the results are notable for mild hypocalcemia (8.4 mg/dL; normal 8.9–10.3 mg/dL) and hypertriglyceridemia (500 mg/dL; normal <200 mg/mL) with increased VLDL. Total cholesterol (170 mg/dL; normal <200 mg/dL), LDL cholesterol (100 mg/dL; normal <130 mg/dL), and HDL (34 mg/dL; normal 34–86 mg/dL) are within normal ranges. The patient is put on a low-fat diet and given a prescription for gemfibrozil.

Peroxisomes are single-membrane organelles that accomplish β-oxidation of long and very long chain fatty acids similar to the mitochondrial β-oxidation pathway. One notable difference of the peroxisome pathway is that peroxisomes generate hydrogen peroxide from fatty acid oxidation. They also conduct oxidation of branched fatty acids and ω-oxidation of ordinary fatty acids. Gemfibrozil is a hypolipidemic drug that is prescribed to treat certain types of hyperlipoproteinemia, notably elevated blood triglyercides with normal cholesterol and LDL; it acts by stimulating proliferation of peroxisomes and increasing gene expression of lipoprotein lipase, resulting in the induction of the fatty acid oxidation pathway in these organelles.

Bridge to Pathology

Zellweger syndrome is a genetic disease caused by a mutation in any one of several genes (locus heterogeneity) involved in peroxisome biogenesis.

- Characterized by a deficiency of peroxisomes which causes an accumulation of very long chain fatty acids and several unusual fatty acids (e.g., hydroxylated and branched fatty acids)
- Mechanism is a defect in fatty acid efflux from peroxisomes
- Most common features are enlarged liver, high blood levels of Cu and Fe, and vision problems
- In affected infants, there is failure to grow, mental retardation, abnormal muscle tone, and multiple developmental abnormalities; infants usually die within first year

Control of Gluconeogenesis by Response Elements

High-Yield

An example of how response elements affect metabolism can be seen in the pathway of gluconeogenesis below.

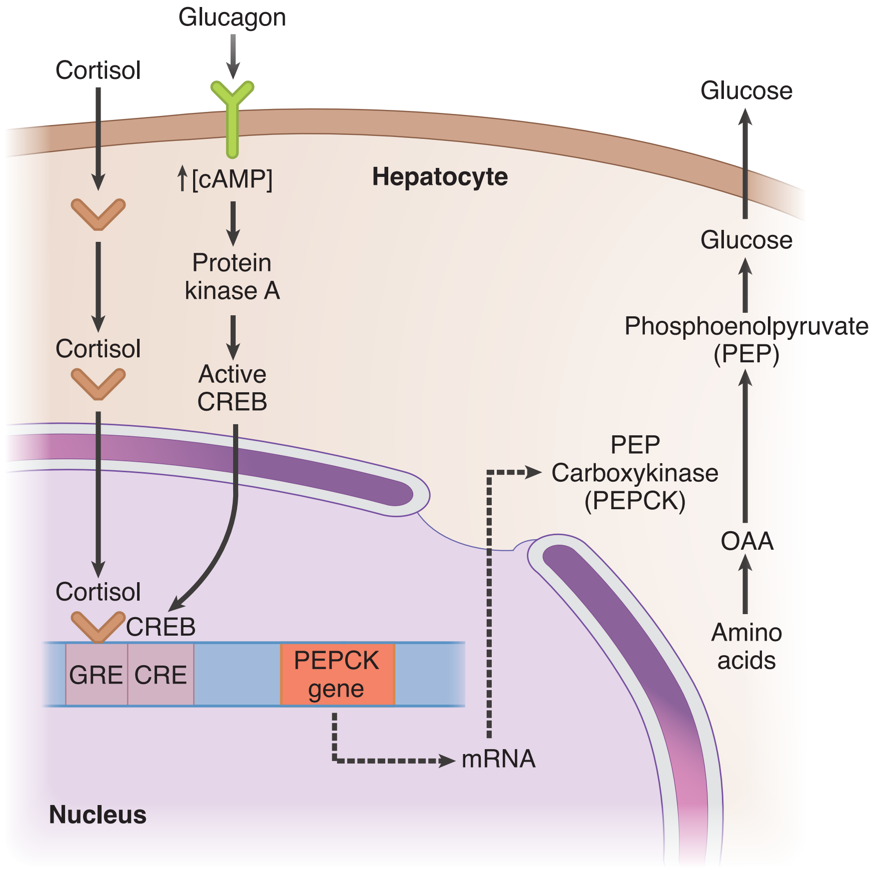


Figure I-5-4. Cortisol and Glucagon Stimulate Gluconeogenesis through Enhancer Mechanisms

Gluconeogenesis is a hepatic pathway whose major function is to maintain adequate glucose in the blood for tissues such as the nerves (brain) and red blood cells during fasting. It also provides glucose during periods of stress. Hormones that activate the pathway include:

- Glucagon secreted in response to hypoglycemia and functioning via a membrane-associated receptor that increases cAMP concentration
- Cortisol secreted in response to stress; permissive for glucagon in hypoglycemia and acts through an intracellular receptor, which, like other steroid receptors, is a zinc-finger DNA binding protein

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes a critical reaction in gluconeogenesis, which under many conditions is the rate-limiting step in the pathway. A cAMP response element (CRE) and a glucocorticoid response element (GRE) are each located upstream from the transcription start site.

Cortisol induces PEPCK gene expression by the following sequence:

- Cortisol diffuses into the hepatocyte, where it
- Binds to its receptor.
- The complex enters the nucleus, and
- Binds (through the zinc fingers) to the glucocorticoid response element (GRE) associated with the PEPCK gene, which
- Increases gene expression.
- PEPCK concentration increases in the cell.
- The rate of gluconeogenesis increases.

Glucagon induces PEPCK gene expression by the following sequence:

- Glucagon binds to a receptor in the cell membrane (Chapter 9).
- cAMP concentration increases.
- Protein kinase A becomes active, and then
- Phosphorylates and activates CREB.
- Activated CREB enters the nucleus and binds to the CRE associated with the PEPCK gene, which
- Increases gene expression.
- PEPCK concentration increases in the cell.
- The rate of gluconeogenesis increases.

These effects of CREB and the cortisol-receptor complex are not entirely independent of each other. Each contributes, along with several other transcription factors, to assembling a complex of activator proteins that ultimately determine the level of PEPCK gene expression.

Recall Question

Which of the following protein classes is related to the transcription factor for steroid receptors?

A. Helix-turn-helix

B. Leucine zipper

C. Rel domains

D. Zinc fingers

Answer: D

Control of Cell Differentiation by Homeodomain Proteins During Development *In Utero*

High-Yield

Sequential and coordinated gene expression is necessary for proper tissue and cell differentiation during embryonic life. Groups of regulatory proteins called homeodomain proteins are major factors in controlling this embryonic gene expression. Each regulatory protein is responsible for activating a different set of genes at the proper time in development.

The regulatory proteins themselves are encoded by genes called homeobox (HOX) or homeotic genes. Another closely related set of genes is the PAX (paired-box) genes. Mutations in HOX or PAX genes might be expected to produce developmental errors. Klein-Waardenburg syndrome (WS-III) is one such developmental disorder resulting from a mutation in a PAX gene.

Clinical Correlate

All of the tissues affected in **Klein-Waardenburg syndrome** are derived from embryonic tissue in which PAX-3 is expressed. Symptoms include:

- Dystopia canthorum (lateral displacement of the inner corner of the eye)
- Pigmentary abnormalities (frontal white blaze of hair, patchy hypopigmentation of the skin, heterochromia irides)
- Congenital deafness
- Limb abnormalities

Co-Expression of Genes

Most eukaryotic cells are diploid, each chromosome being present in two homologous copies. The alleles of a gene on the 2 homologous chromosomes are usually co-expressed. In a person heterozygous for the alleles of a particular gene, for example a carrier of sickle cell trait, two different versions of the protein will be present in cells that express the gene. In the person heterozygous for the normal and sickle alleles, about 50% of the β-globin chains will contain glutamate and 50% valine at the variable position (specified by codon 6).

Major exceptions to this rule of codominant expression include genes:

- On the Barr body (inactivated X chromosome) in women
- In the immunoglobulin heavy and light chain loci (ensuring that one B cell makes only one specificity of antibody)
- In the T-cell receptor loci

Clinical Correlate

Holoprosencephaly (HPE) is a common developmental anomaly of the human forebrain and midface, where the cerebral hemispheres fail to separate into distinct left and right halves. Haploinsufficiency for **Sonic Hedgehog (*SHH*)** is a cause of HPE.

Other Mechanisms for Controlling Gene Expression in Eukaryotes

Some of the mechanisms which control gene expression in eukaryotic cells are described below.

Table I-5-2. Control of Eukaryotic Gene Expression and Protein Levels

Control Point	Example
Inactivation of specific chromo somes or chromosomal regions during development	One X chromosome in each cell of a woman is inactivated by condensation to heterochromatin (Barr bodies)
Local chromatin-modifying activities	Acetylation of histones increases gene expression (many genes) Methylation of DNA silences genes in genetic imprinting (Prader-Willi and Angelman syndromes)
Gene amplification	Many oncogenes are present in multiple copies: *erbB* amplified in certain breast cancers Dihydrofolate reductase genes are amplified in some tumors, leading to drug resistance
Specific transcription factors	Steroid hormone receptors, CREB, and homeodomain proteins
Processing mRNA	Alternative splicing of mRNA in the production of membrane-bound vs. secreted antibodies
Rate of translation	Heme increases the initiation of β-globin translation
Protein modification	Proinsulin is cleaved to form active insulin
Protein degradation rate	ALA synthase has a half-life of 1 hour in the hepatocyte

Bridge to Medical Genetics

Genetic Imprinting in Prader-Willi Syndrome

Genetic imprinting of a few gene regions results in monoallelic expression. In some cases, this imprinting is according to the parent of origin. The gene involved in Prader-Willi syndrome is on chromosome 15 and is imprinted so that it is normally expressed only from the paternal, not the maternal, chromosome.

In such a case, if one inherits a paternal chromosome in which this region has been deleted, Prader-Willi syndrome results. It can also result from uniparental (maternal) disomy of chromosome 15. Symptoms of Prader-Willi include:

- Childhood obesity and hyperphagia
- Hypogonadotrophic hypogonadism
- Small hands and feet
- Mental retardation
- Hypotonia

Review Questions

Select the ONE best answer.

1. Klein-Waardenburg syndrome is a single-gene disorder that includes dystopia canthorum (lateral displacement of the inner corner of the eye), impaired hearing, and pigmentary abnormalities. The gene involved is most likely to be a

   A. pseudogene
   B. proto-oncogene
   C. transgene
   D. homeotic gene
   E. tumor suppressor gene

2. Enhancers are transcriptional regulatory sequences that function by enhancing the activity of

   A. general transcriptional factors
   B. RNA polymerase to enable the enzyme to transcribe through the terminating region of a gene
   C. transcription factors that bind to the promoter but not to RNA polymerase
   D. RNA polymerase at a single promoter site
   E. spliceosomes

3. A pharmacologist employed by a pharmaceutical company is investigating the mechanism of action of a new drug that significantly inhibits the division of tumor cells obtained from patients with acute myelogenous leukemia. He has determined that the drug serves as a potent inactivator of chromatin-modifying activity that up-regulates the expression of a cluster of oncogenes in these tumor cells. Which type of chromatin-modifying activity is most likely stimulated by the enzyme target of this drug?

   A. Acetylation of core histones
   B. Binding of histone H1 to nucleosomes
   C. Deacetylation of core histone H4
   D. Deamination of cytosine bases in DNA
   E. Methylation of cytosine bases in DNA

Answers

1. **Answer: D.** Multiple developmental abnormalities due to mutation in a single gene.

2. **Answer: D.** Specific transcription factors (e.g., any steroid receptor) bind to specific DNA sequences (enhancers) and to RNA polymerase at a single promoter sequence and enable the RNA polymerase to transcribe the gene more efficiently.

3. **Answer: A.** Acetylation of nucleosome core histones is strongly associated with transcriptionally active chromatin. Other modifications **(choices B, C and E)** are associated with down-regulation of gene expression. Deamination of cytosine in DNA **(choice D)** is not related to chromatin remodeling and increased gene expression.

Genetic Strategies in Therapeutics 6

Learning Objectives

- ❑ Understand concept and applications of restriction endonucleases
- ❑ Answer questions about medical applications of recombinant DNA

RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology allows a DNA fragment from any source to be joined *in vitro* with a nucleic acid vector that can replicate autonomously in microorganisms. This technology has several functions:

- Provides a means of analyzing and altering genes and proteins
- Provides the reagents necessary for genetic testing for carrier detection and prenatal diagnosis of genetic diseases and for gene therapy
- Provides a source of a specific protein (e.g., recombinant human insulin) in almost unlimited quantities

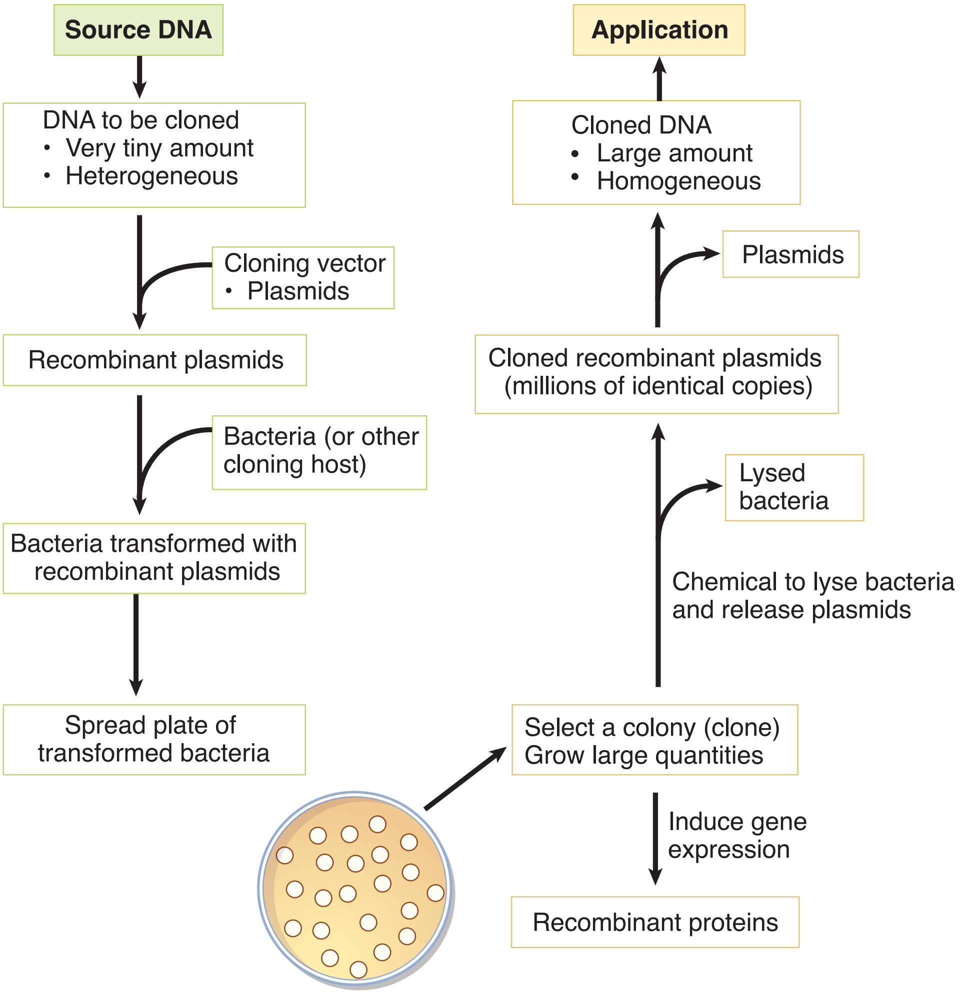


Figure I-6-1. Cloning Recombinant DNA

The DNA to be cloned is usually present in a small quantity and is part of a heterogeneous mixture containing other DNA sequences. The goal is to produce a large quantity of homogeneous DNA for one of the above applications. The general strategy for cloning DNA and isolating the cloned material can be seen above. The steps include:

- Ligate the DNA into a piece of nucleic acid (the vector) that can be autonomously replicated in a living organism. The vector containing the new DNA is referred to as a recombinant vector.
- Transfer the recombinant vectors into host cells.
- Grow the host cells in isolated colonies so that each colony contains only one recombinant vector.
- Select a colony for study.
- Grow a large quantity of that colony.
- Lyse the host cells and re-isolate the replicated recombinant vectors.
- Remove (by restriction enzyme cutting) the cloned DNA from the vector.

Note

Each cultured colony is a clone; all members are genetically identical.

CLONING RESTRICTION FRAGMENTS OF DNA: THE HUMAN GENOME PROJECT

The Human Genome Project, initiated in 1991, involved the identification of the entire 3 billion–base-pair human DNA sequence. This project has now been completed. Although humans appear to be quite different from each other, the sequence of our DNA is, in reality, highly conserved. On average, 2 unrelated individuals share over 99.9% of their DNA sequences. For the Human Genome Project, DNA was obtained from a relatively small number of individuals. Although the variety of techniques involved is well beyond this review, the basic effort required cloning DNA restriction fragments, determining their sequences, and identifying overlaps to align the fragments properly. The major points to be understood include:

- Restriction endonucleases cut DNA specifically at palindrome sequences, yielding restriction fragments of chromosomes.
- The restriction fragments are cloned in vectors.
- The cloned fragments are re-isolated from the cloned recombinant vectors.
- The restriction fragments from each clone are sequenced.

Note

Human Genome Project data can be used to identify:

- Protein-coding genes
- Regulatory sequences in noncoding DNA
- Polymorphic genetic markers (restriction endonuclease sites, short tandem repeats, and single nucleotide polymorphisms) dispersed throughout chromosomes in coding and noncoding DNA

Producing Restriction Fragments: Restriction Endonucleases

High-Yield

Chromosomes obtained from the DNA donors were cut with restriction endonucleases to produce restriction fragments. These enzymes are isolated from bacteria, their natural source. There are many different restriction endonucleases isolated from a variety of bacteria that are now readily available commercially. In bacteria, they act as part of a restriction/modification system that protects the bacteria from infection by DNA viruses.

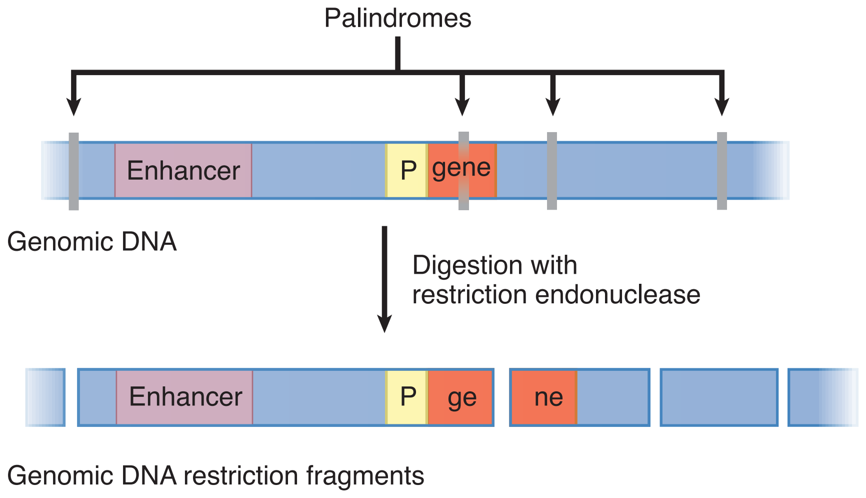


Figure I-6-2. DNA Digestion with a Restriction Endonuclease

Restriction endonucleases recognize double-stranded DNA sequences called palindromes (inverted repeats) usually of 4 to 8 base pairs in length. For example, Figure I-6-3 shows the recognition site for *Eco*RI, a restriction endonuclease isolated from *Escherichia coli.* A palindrome can be identified by examining the sequence of only one strand. Draw a line through the center of the sequence (through the central base for palindromes with an odd number of nucleotides). If the sequence is folded along this line, the bases should pair.

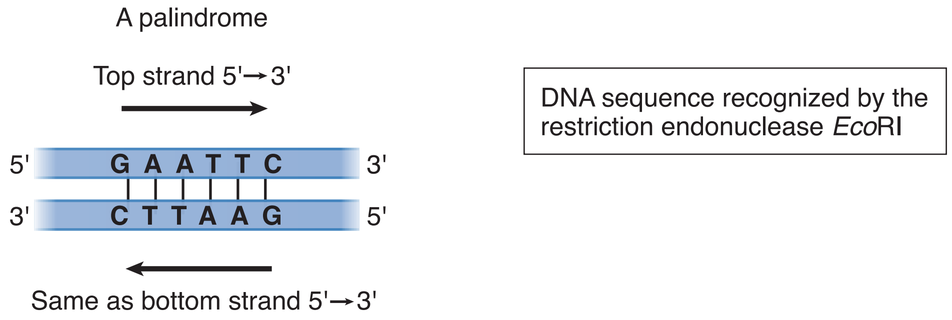


Figure I-6-3. EcoRI Recognition Sequence

DNA from a source to be cloned is mixed with a particular restriction endonuclease, such as *Eco*RI, producing DNA restriction fragments. Some restriction endonucleases, such as *Eco*RI, produce offset cuts within the palindrome, yielding "sticky ends" on the fragments. Sticky ends are advantageous in facilitating the recombination of a restriction fragment with the vector DNA.

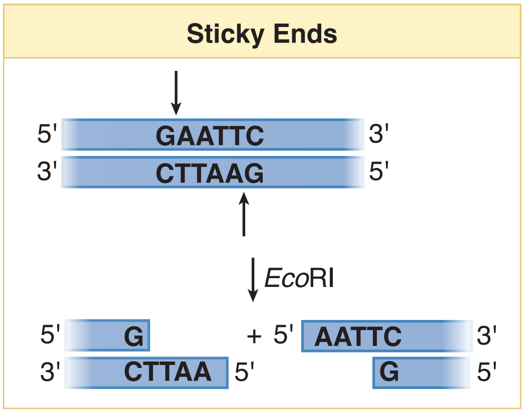


Figure I-6-4. Example of a Restriction Endonuclease

Cloning Restriction Fragments Using Vectors

Each restriction fragment (which may include a gene) must be inserted into a vector to be cloned. A vector is a piece of DNA (plasmid, viral chromosome, yeast chromosome) capable of autonomous replication in a host cell—for instance, the plasmid pBR322 shown below. The DNA used as a vector usually has:

- At least one type of palindrome recognized by a restriction endonuclease
- An origin for autonomous replication
- At least one gene for resistance to an antibiotic (allows selection for colonies with recombinant plasmids)

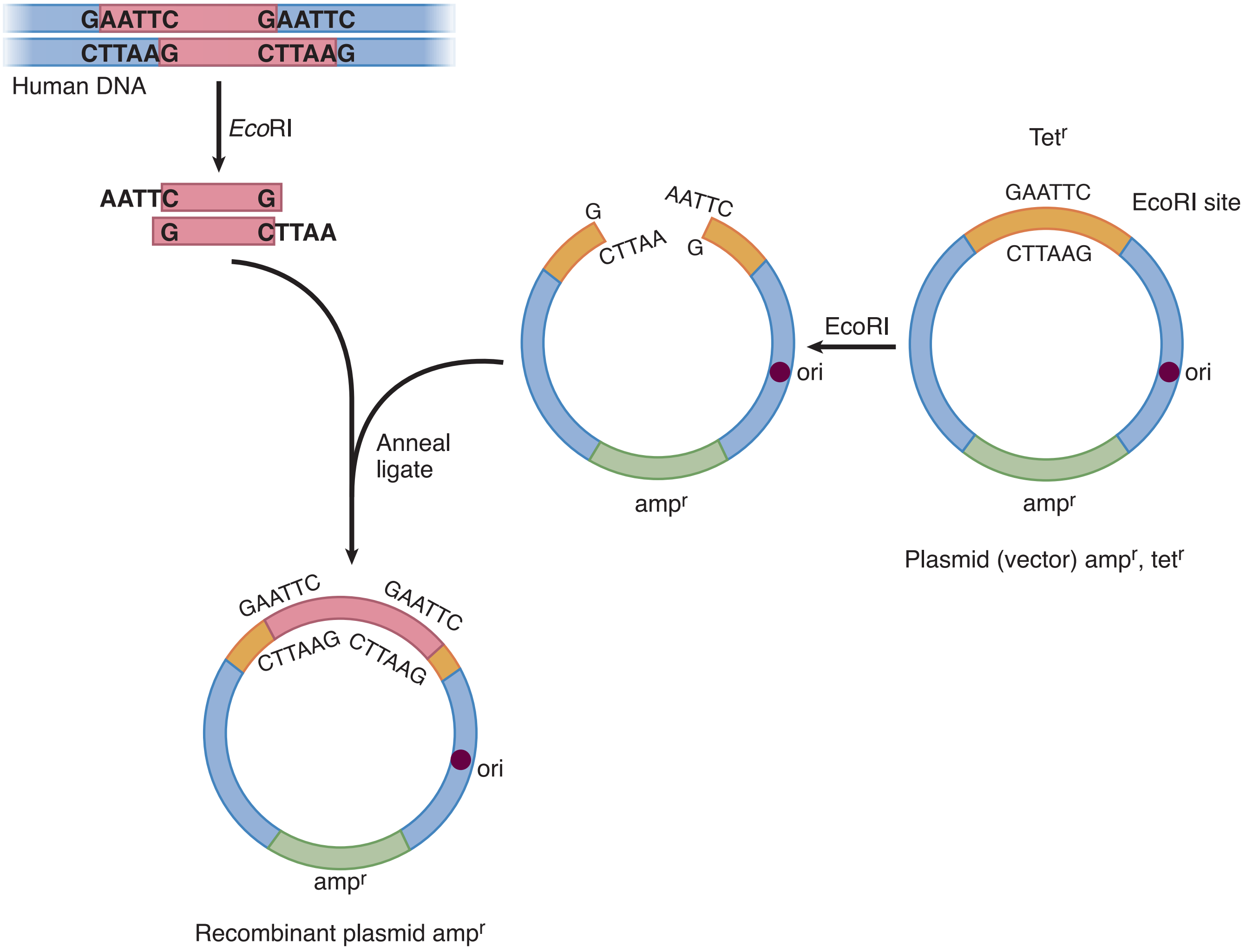


Figure I-6-5. Formation of a Recombinant Plasmid

Genomic Libraries

Genomic libraries have been used to sequence DNA as accomplished in the Human Genome Project. These sequences can be used to identify:

- Protein-coding genes
- Restriction endonuclease sites (see Margin Note: Restriction Maps)
- Other genetic markers (short tandem repeats, single nucleotide polymorphisms)
- Non-expressed DNA (enhancers, promoters, introns, noncoding DNA between gene regions)

Bridge to Medical Genetics

Restriction Maps

- Line drawings of DNA identifying sites cut by restriction endonucleases
- Identify potential RFLP markers for genetic diagnosis

CLONING GENES AS cDNA PRODUCED BY REVERSE TRANSCRIPTION OF CELLULAR mRNA

If the end goal of cloning is to have a cloned gene expressed in a cell, the entire coding sequence must be cloned intact. Furthermore, if a cloned eukaryotic gene is to be expressed in bacteria (to make recombinant proteins), the gene must not contain introns, which could not be processed in a prokaryotic cell. In these cases, it is more convenient to clone cDNA rather than DNA restriction fragments.

Producing cDNA by Reverse Transcription of mRNA

Cytoplasmic mRNA is isolated from a cell known to express the desired gene. Reverse transcriptase, along with other components (Figure I-6-6), is used *in vitro* to produce double-stranded cDNA that is subsequently recombined with a chosen vector to produce the recombinant DNA for cloning. In this approach:

- All genes expressed will be cloned along with the desired gene.
- None of the non-expressed DNA in the cell will be cloned.
- Each cDNA represents the complete coding sequence of a gene.
- The cDNAs have no introns.
- An expression library is produced at the end of the cloning procedure.

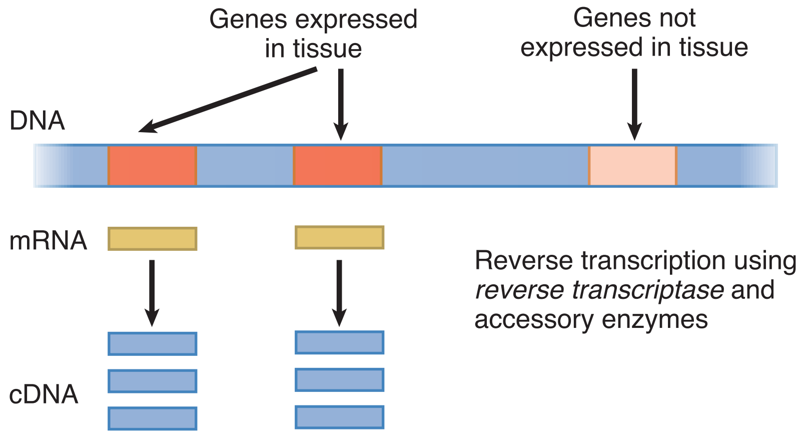


Figure I-6-6. Cloning Expressed Genes by Producing cDNAs

cDNA (Expression) Libraries

Once the recombinant expression vectors containing the cDNA inserts are produced, they are cloned in bacteria (or other host cells) and produce cDNA (expression) libraries. These libraries can be used to:

- Sequence specific genes and identify disease-causing mutations
- Produce recombinant proteins (insulin, factor VIII, HBsAg for vaccination)
- Conduct gene replacement therapy
- Produce transgenic animals

Several of these applications are discussed in more detail in subsequent sections of this chapter.

Table I-6-1. Comparison of Genomic and cDNA (Expression) Libraries

	Genomic Libraries	cDNA (Expression) Library
Source of DNA	Chromosomal DNA	mRNA (cDNA)
Enzymes to make library	Restriction endonuclease DNA ligase	Reverse transcriptase DNA ligase
Contains nonexpressed sequences of chromosomes	Yes	No
Cloned genes are complete sequences	Not necessarily	Yes
Cloned genes contain introns	Yes	No
Promoter and enhancer sequences present	Yes, but not necessarily in same clone	No
Gene can be expressed in cloning host (recombinant proteins)	No	Yes
Can be used for gene therapy or constructing transgenic animals	No	Yes

MEDICAL APPLICATIONS OF RECOMBINANT DNA

Recombinant DNA can be used as follows:

- To produce recombinant proteins, used variously in:
  - Replacement therapy (e.g., insulin in diabetes)
  - Disease prevention (e.g., vaccines)
  - Diagnostic tests (e.g., monoclonal antibodies)
- To conduct gene therapy in the treatment of genetic diseases

Recombinant Proteins

High-Yield

Recombinant proteins can be made by cloning the relevant gene for the protein in a host organism, growing large quantities of the organism, and inducing it to express the gene (as indicated in the lower right of Figure I-6-1). Many therapeutics proteins are now mass-produced as recombinant proteins.

Table I-6-2. Examples of Protein Products of Recombinant DNA Technology

Product	Produced in	Use
Insulin	*E. coli*	Diabetes
Growth factor	*E. coli*	Growth defects
Epidermal growth factor	*E. coli*	Burns, ulcers
Hepatitis B vaccine	*Saccharomyces cerevisae*	Prevention of viral hepatitis
Erythropoietin	Mammalian cells	Anemia
Factor VIII	Mammalian cells	Hemophilia

Note

- **Ex vivo**: cells modified outside the body, then transplanted back in
- **In vivo**: gene changed in cells still in body

Gene Therapy

High-Yield

Gene therapy now offers potential cures for individuals with inherited diseases. The initial goal is to introduce a normal copy of the gene that is defective into the tissues that give rise to the pathology of the genetic disease.

For instance, about 50% of children with severe combined immunodeficiency have a mutation in the gene encoding the γ chain common to several of the interleukin receptors. Recently, cDNA from a normal γ-chain gene was used to transduce autologous cells from infants with X-linked severe combined immunodeficiency (SCID) with subsequent correction of the defects in their T cells and natural killer cells.

- Gene transfer requires a delivery vector (retrovirus, adenovirus, liposome).
- Only tissues giving rise to the disease pathology are targeted for gene therapy.
- The normal gene is not inherited by offspring.

Gene delivery vectors

For gene replacement therapy to be a realistic possibility, efficient gene delivery vectors must be used to transfer the cloned gene into the target cells' DNA. Because viruses naturally infect cells to insert their own genetic material, most gene delivery vectors now in use are modified viruses. A portion of the viral genome is replaced with the cloned gene (as either DNA or RNA) such that the virus can infect but not complete its replication cycle. Steps in the production of a retrovirus for gene replacement therapy are illustrated in Figure I-6-7.

Retroviruses and adenoviruses were early vectors used in gene delivery. However, newer strategies take advantage of vectors with better properties, such as adeno-associated viruses (AAV). Major advantages of AAV include having no disease association in humans and limited innate immunity. In addition, AAV restricts expression to specific tissues: A tissue-specific promoter in the AAV is genetically engineered to control transcription of the inserted transgene.

Table I-6-3. Vectors Used in Gene Therapy

Viral Vector	Retroviruses	Adenovirus	Adeno-Associated Virus (AAV)
Family	Retroviridae	Adenoviridae	Parvoviridae
Genome	ssRNA	dsDNA	ssDNA
Disease association?	Yes	Yes	No
Inserts into chromosome?	Yes	No	No (episomal)
Innate immunity?	Yes	Yes	Limited

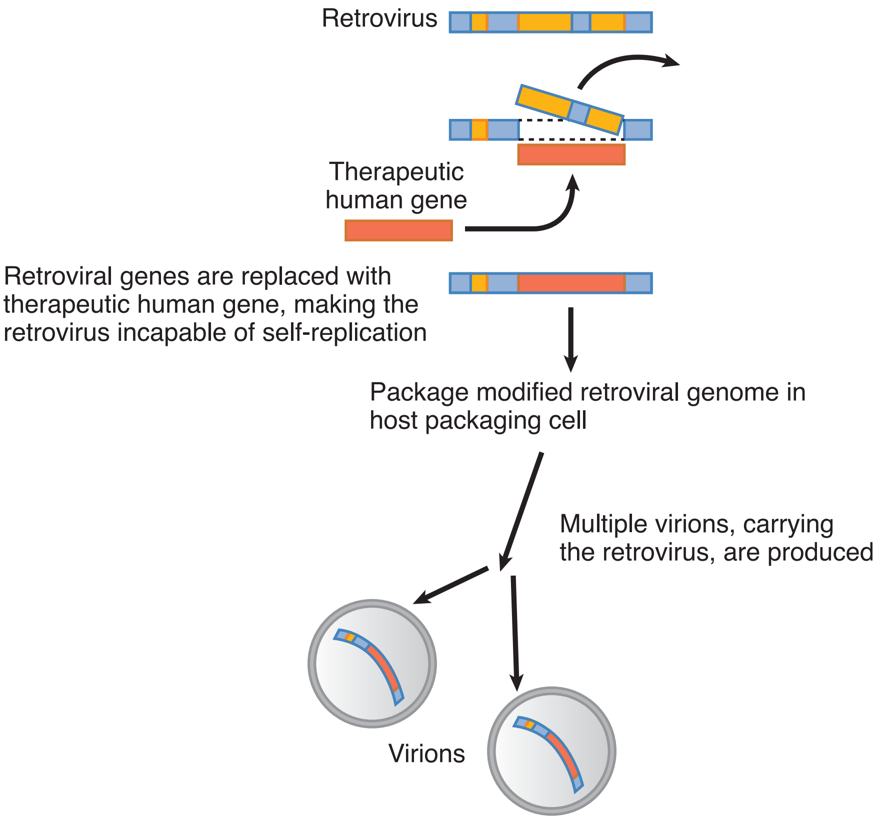


Figure I-6-7. Preparation of a Retrovirus for Gene Replacement Therapy

There are 2 strategies for delivering a therapeutic gene (transgene) into an individual.

- ***In vivo*** gene replacement therapy involves the direct delivery of a therapeutic gene into a patient's body. Upon entry into the target cells, the inserted transgene is expressed into a therapeutic protein.
- ***Ex vivo*** gene replacement therapy involves the genetic manipulation of a patient's target cells outside the body. Target cells are infected with a recombinant virus harboring the therapeutic transgene. The genetically modified target cells, harboring and expressing the therapeutic protein, are then reintroduced into the same patient.

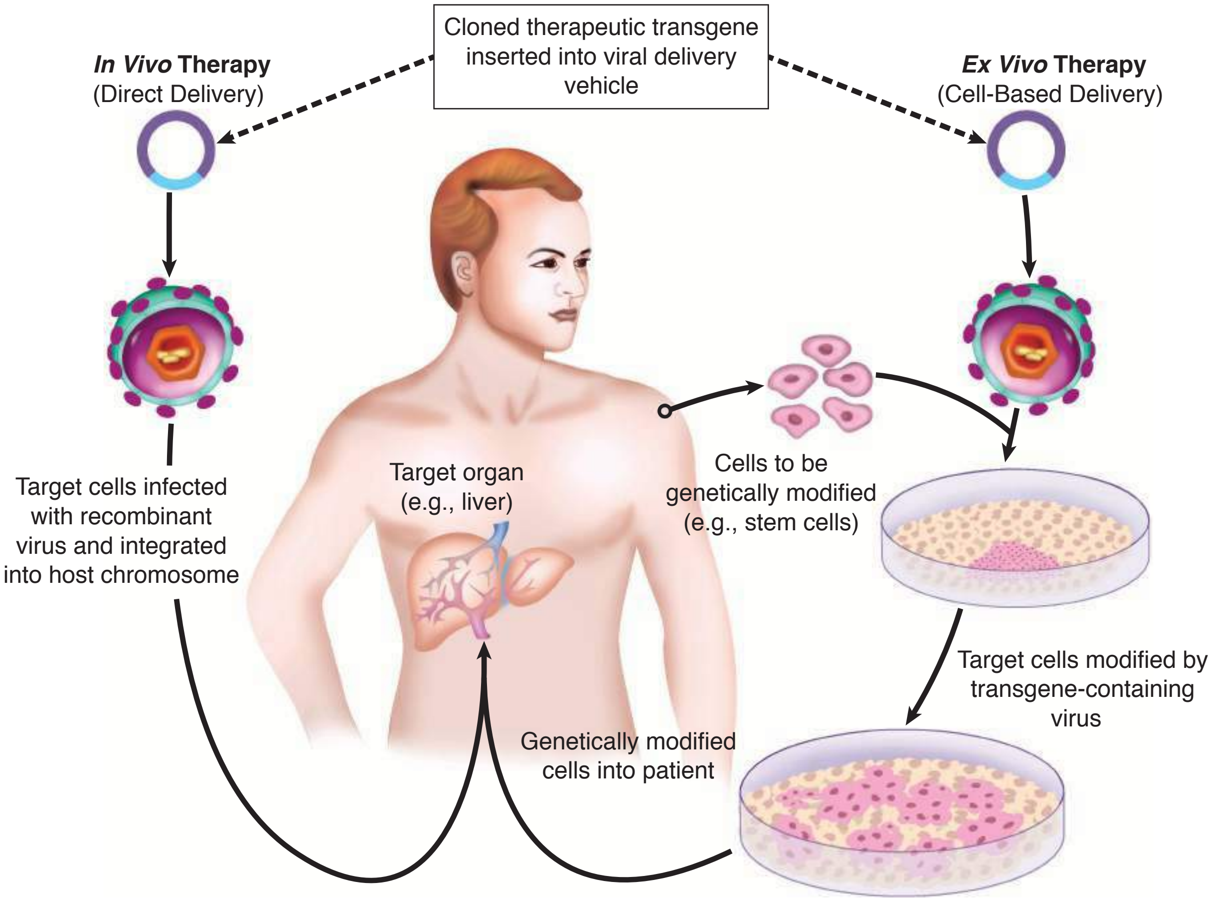


Figure I-6-8. In Vivo and Ex Vivo Gene Replacement Therapies

Gene replacement therapy (*in vivo* therapy) for cystic fibrosis illustrates an important example of direct delivery of a transgene.

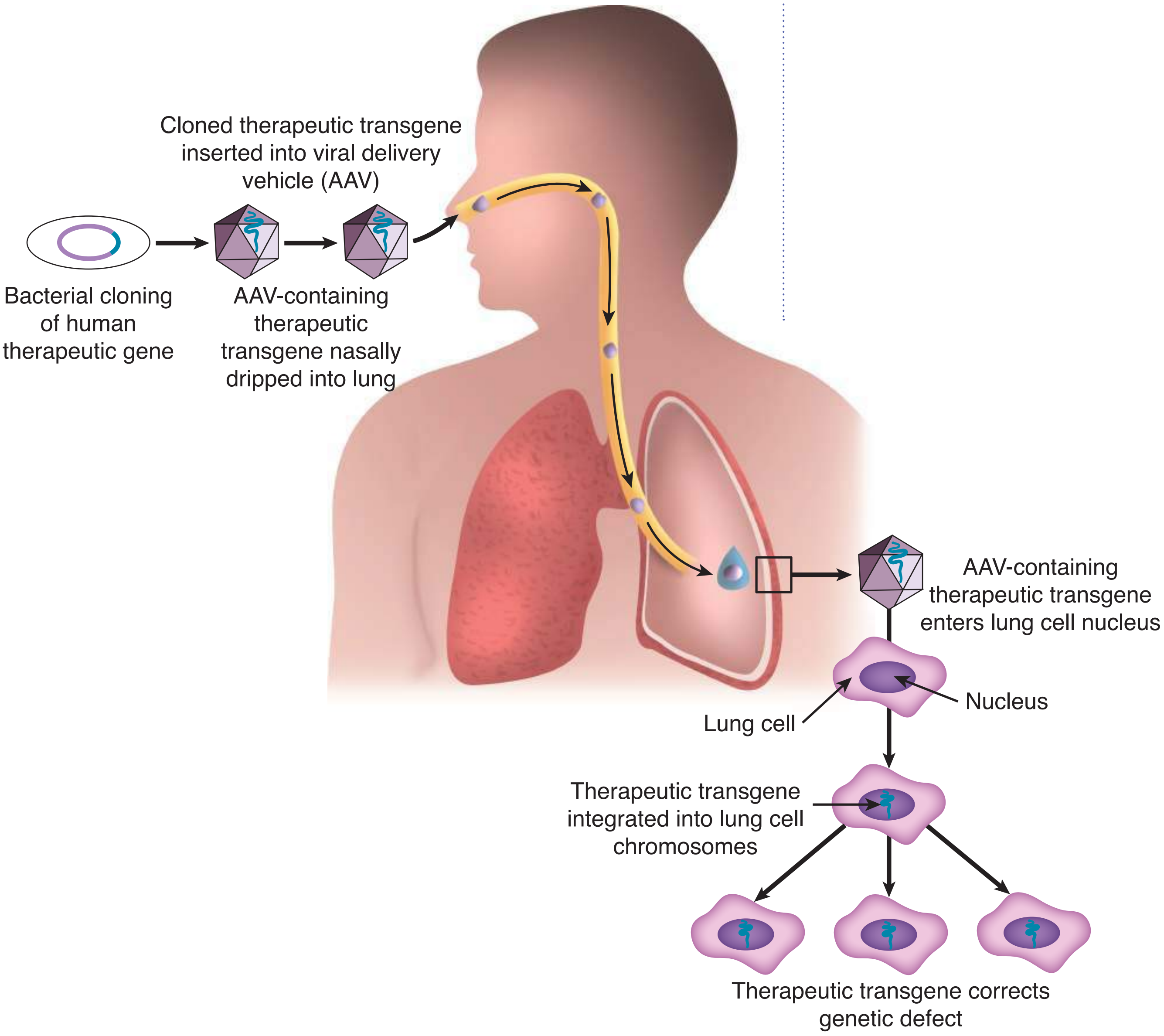


Figure I-6-9. Gene Replacement Therapy for Cystic Fibrosis

An important example of *ex vivo* gene replacement therapy is illustrated below.

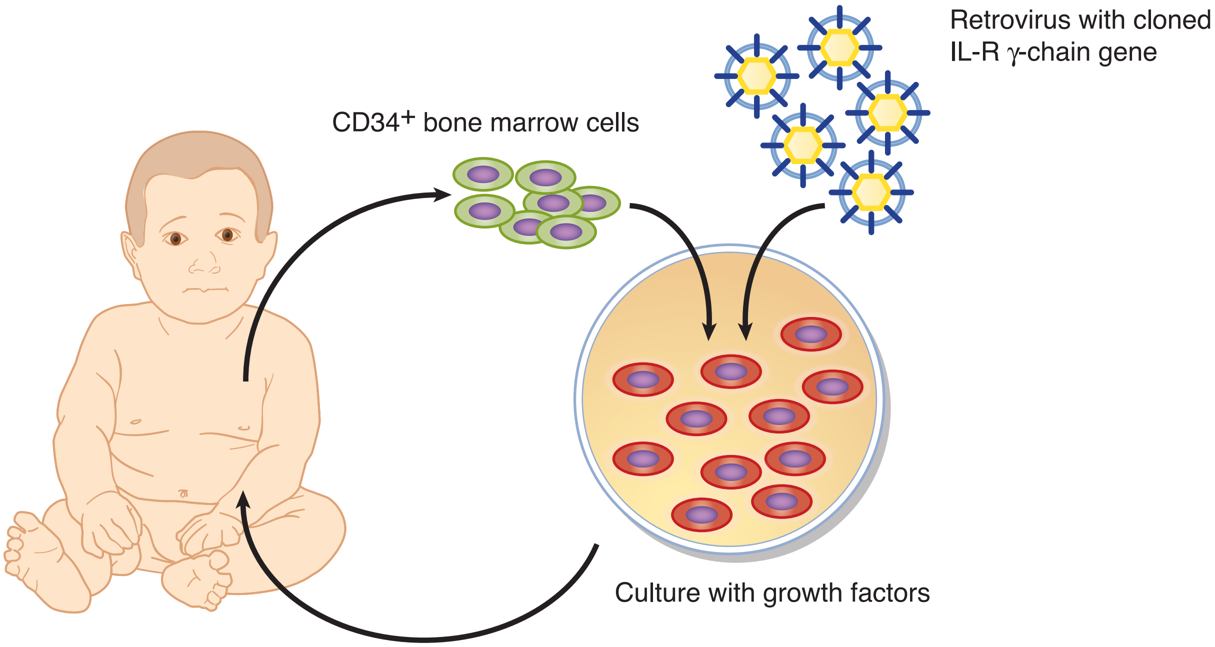


Figure I-6-10. Ex Vivo Gene Replacement Therapy for X-Linked Severe Combined Immunodeficiency

Remaining challenges to gene replacement therapy

Although much progress has been made in gene replacement therapy, significant challenges still remain. These challenges include:

- Targeting the therapeutic gene to the appropriate tissues
- Low-level or transient expression of the therapeutic gene
- Problems caused by random insertion of the therapeutic gene into the host DNA.

Recall Question

Which of the following is true for both genomic libraries and cDNA (expression) libraries?

A. Cloned genes contain introns

B. DNA ligase is used

C. Reverse transcriptase is used

D. Can be used for gene therapy

Answer: B

RNA Interference

RNA interference (RNAi) refers to downregulation of gene expression through the use of small RNA molecules, which mediate gene expression by either inhibiting translation or causing premature degradation of the genes' mRNAs. Two types of small RNA molecules are involved in RNA interference: microRNA (miRNA) and small interfering RNA (siRNA). The RNAi pathway occurs in many eukaryotes, including humans, and plays a central role in defending cells against viruses and transposons (discussed in Microbiology Lecture Notes).

The RNAi process begins when an enzyme known as dicer cleaves long double-stranded RNA into small double-stranded RNA fragments (siRNA) approximately 20 nucleotides in length. The double-stranded siRNA then unwinds into two single-stranded RNAs: the passenger strand (sense strand), which subsequently degrades, and the guide strand (antisense strand), which associates into the RNA-induced silencing complex (RISC). Next, the guide strand pairs with a complementary sequence in a messenger RNA molecule and induces cleavage using argonaute, the catalytic component of the RISC complex. Since the mRNA is degraded, its encoded protein is not produced. The result is posttranscriptional gene silencing. This effect is often referred to as "knockdown" because gene expression continues, though in greatly reduced extent. In "knockout," by contrast, gene expression is entirely absent.

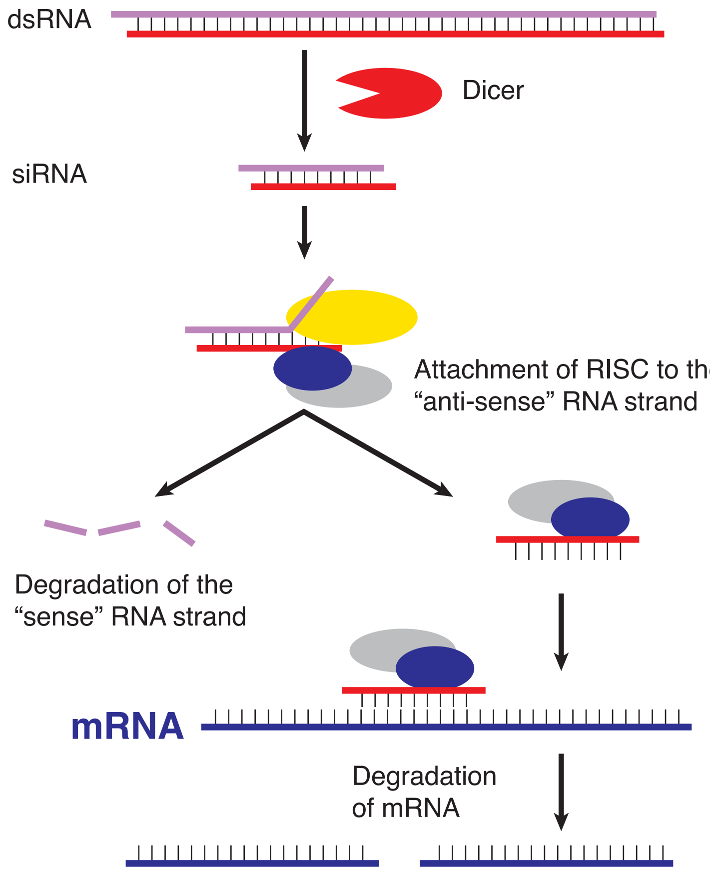


Figure I-6-11. RNAi Pathway

To time-limit the effects of RNAi, siRNAs can be administered as stabilized RNAs conjugated to targeting compounds or enclosed in lipid vesicles. A single RNAi treatment can silence expression of a particular gene for 2 weeks. siRNAs can be stabilized against endogenous RNases in blood or cells by modifying the 2' hydroxyl with a methyl or fluorine group.

RNA interference technology is being explored as a treatment for cancer and many neurodegenerative diseases and for use in antiviral therapies. Clinical trials are also exploring the use of RNAi in other clinical applications, such as therapy for age-related macular degeneration.

Table I-6-4. Summary of Important Points About Recombinant DNA

Restriction endonucleases	Recognize palindromes in dsDNA: 5′ - - - G A A T T C - - - 3′ 3′ - - - C T T A A G - - - 5′ Cut leaving sticky ends: 5′ - - - G A A T T C - - - 3′ 3′ - - - C T T A A G - - - 5′ Used to make restriction maps of DNA Produce fragments for genetic analysis Produce fragments for making recombinant DNA and cloning DNA sequences
Vectors for recombinant DNA and cloning	Plasmid: • Restriction site • Replication origin • Resistance to antibiotic(s) Expression vector also requires: • Promoter • Shine-Dalgarno sequence Other vectors: phage, YACs
Approaches to cloning DNA	Genomic DNA • Restriction endonucleases fragment DNA • Total nuclear DNA cloned • Genes contain introns cDNA • Reverse transcription of mRNAs from cell • Genes expressed cloned • Genes have no introns
Uses of cloned genes	Produce recombinant proteins Gene therapy (somatic) Transgenic animals (germline) Produce cDNA probes for blots

Review Questions

1. If a patient with cystic fibrosis were to be treated by gene therapy, which type of cells should be targeted as host cells?

   A. Germ cells
   B. Epithelial cells
   C. T cells
   D. Hemopoietic stem cells

2. A pharmaceutical firm is interested in the bacterial production of thymidylate synthase in large quantities for drug-targeting studies. An important step in the overall cloning strategy involves the ligation of synthase cDNA into a plasmid vector containing a replication origin, an antibiotic resistance gene, and a promoter sequence. Which additional nucleotide sequence should be included in this vector to ensure optimal production of the thymidylate synthase?

   A. Operator sequence
   B. PolyA sequence
   C. Shine-Dalgarno sequence
   D. Attenuator sequence
   E. 3′-splice acceptor sequence

3. Restriction fragment length polymorphisms may be produced by mutations in the sites for restriction endonucleases. For instance, a single base change in the site for the nuclear *Sal*I produces the sequence GTGGAC, which can no longer be recognized by the enzyme. What was the original sequence recognized by *Sal*I?

   A. GTAGAC
   B. GCGGAC
   C. CTGGAC
   D. GTCGAC
   E. GTGTAC

Answers

1. **Answer: B.** The pathogenesis of cystic fibrosis is related to defective chloride transport in epithelial cells.

2. **Answer: C.** Incorporation of a Shine-Dalgarno sequence into the expression vector will promote ribosome binding to the translation start site on the mRNA produced by transcription of the cDNA insert.

3. **Answer: D.** All options represent single-base changes in the mutant sequence in the stem, but only choice D reestablishes a palindrome.

Techniques of Genetic Analysis 7

Learning Objectives

- ❏ Interpret scenarios about blotting technique
- ❏ Explain information related to polymerase chain reaction

Techniques of genetic analysis are assuming an increasingly larger role in medical diagnosis. These techniques, which once were a specialized part of medical genetics, are now becoming essential tools for every physician to understand. Blotting techniques allow testing for genetic diseases, gene expression profiling, and routine testing for antigens and antibodies. The polymerase chain reaction (PCR) is now an essential tool in many aspects of genetic testing, forensic medicine, and paternity testing. These techniques are discussed in this chapter, but their applications will be further explored in Medical Genetics (Section II of this book).

BLOTTING TECHNIQUES

Blotting techniques have been developed to detect and visualize specific DNA, RNA, and protein among complex mixtures of contaminating molecules. These techniques have allowed the identification and characterization of the genes involved in numerous inherited diseases.

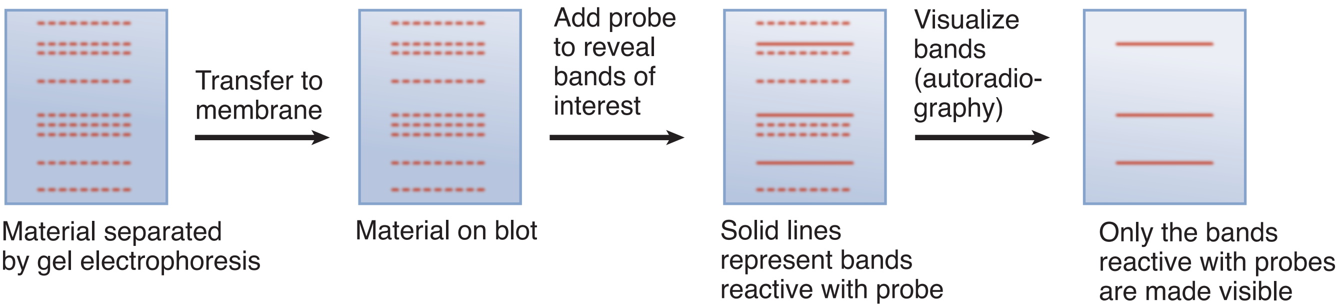


Figure I-7-1. Blotting Technique

The fragments in the material to be analyzed (DNA, RNA, or protein) are separated by gel electrophoresis. The smaller molecules travel faster and appear nearer the bottom of the gel. The bands of material in the gel are transferred, or blotted, to the surface of a membrane. The membrane is incubated with a (usually radioactive) labeled probe that will specifically bind to the molecules of interest. Visualization of the labeled probe (usually by autoradiography) will reveal which band(s) interacted with the probe.

The most common types of blots are compared below. Most typically, DNA restriction fragments are analyzed on a Southern blot.

Table I-7-1. Types of Blots

Blot Type	Material Analyzed	Electro-phoresis Required	Probe Used	Purpose
Southern	DNA	Yes	^{32}P-DNA	To determine which restriction fragments of DNA are associated with a particular gene
Northern	RNA	Yes	^{32}P-DNA	To measure sizes and amounts of specific mRNA molecules to answer questions about gene expression
Western	Protein	Yes	^{125}I- or enzyme-linked antibody	To measure amount of antigen (proteins) or antibody
Dot (slot) (Figure II-6-1)	RNA, DNA, or protein	No	Same as for blots above	To detect specific DNA, RNA, protein, or antibody

Probes

DNA probes are radioactively labeled single-stranded DNA molecules that are able to specifically hybridize (anneal) to particular denatured DNA sequences. Examples include:

- Probes that bind to part of a specific gene region; often produced by cloning cDNA transcribed from the gene and labeling it with ^{32}P, a radioactive isotope of phosphorus
- Probes that bind to markers known to be in close proximity (closely linked) to a gene
- Probes that bind specifically to a single allele of a gene—allele-specific oligonucleotide (ASO) probes (Figure II-6-2)

When protein is separated and analyzed on a Western blot, ^{125}I-labeled antibody specific for the protein of interest is used as a probe.

The probe is an important part of analyzing any blot because the only bands that will appear on the final autoradiogram are those to which the probe has hybridized (see figure below).

DNA probes are used to selectively detect DNA fragments. Staining with ethidium bromide can be used to visualize and detect *all* DNA fragments in a gel, provided the fragments are present in sufficient quantities. Ethidium bromide intercalates between stacked bases and fluoresces when exposed to UV light.

Southern Blots and Restriction Fragment Length Polymorphisms (RFLPs)

High-Yield

Although unrelated individuals share over 99.9% of their DNA sequences, the fact that the human genome contains over 3 billion base pairs means that 2 unrelated individuals' DNA will differ at over a million base pairs (0.1% of a billion equals a million). These differences include mutations in restriction endonuclease sites that can be analyzed as RFLPs on Southern blots.

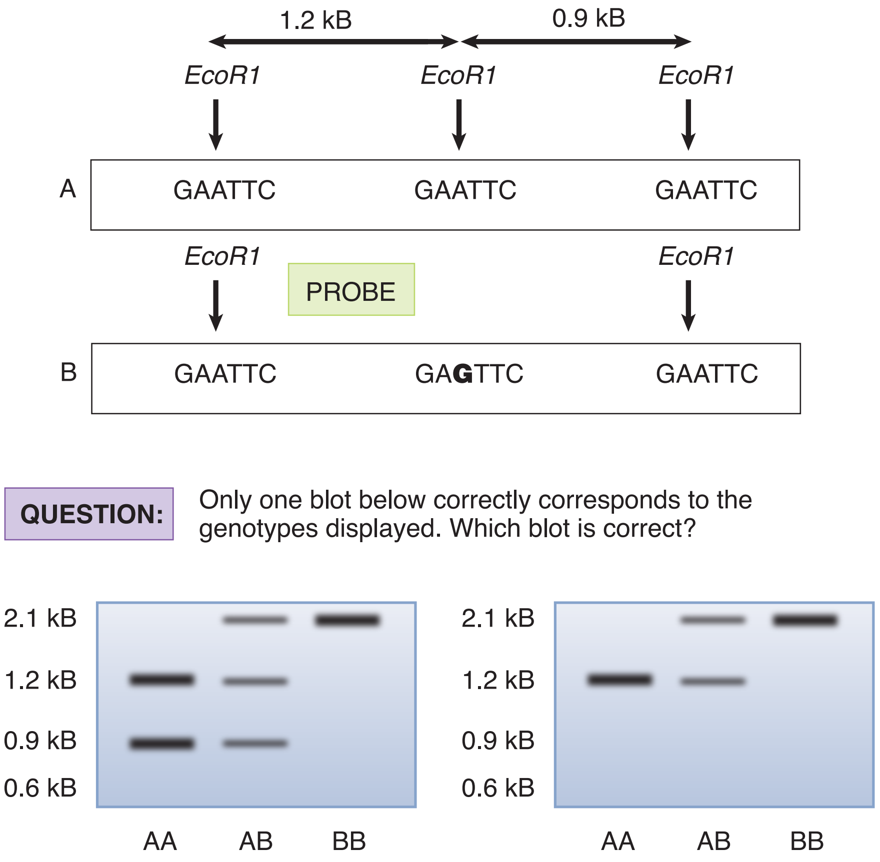


Figure I-7-2. Restriction Fragment Length Polymorphism Analysis on a Southern Blot

A pair of homologous chromosomes is shown above. They are designated chromosomes A and B. The figure shows the same region on both chromosomes and identifies sites (GAATTC) cut by the restriction endonuclease EcoR1. The probe used on the Southern blot binds to the area of the chromosomes indicated in the diagram. DNA samples from 3 individuals are tested: AA (homozygous for A at this region), AB (heterozygous at this region), and BB (homozygous for B at this region). At the bottom, the figure also presents 2 blots, only one of which correctly represents the results seen on the autoradiogram. Which blot is correct?

(Answer: The blot on the right is correct.)

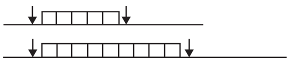
Repetitive sequences known as VNTRs (variable number of tandem repeats) make some contribution to RFLPs, predominantly in the centromeric and telomeric regions of chromosomes (*see* margin note: VNTR Sequences and RFLP).

Note

VNTR Sequences and RFLP

Variable number of tandem repeat (VNTR) sequences contribute to some restriction fragment length polymorphisms (RFLPs). A VNTR sequence designates a unit of nucleotides usually between 15 and 60 bp that is repeated in tandem multiple times at a particular location in the DNA. Although the repeated sequence is shared by all individuals, the number of repeated units is variable from person to person.

These VNTR sequences (boxes) can be flanked by restriction endonuclease sites (arrows). The probe used to detect the RFLP would bind to the repeated unit.



RFLPs and genetic testing

RFLPs may be used in genetic testing to infer the presence of a disease-causing allele of a gene in a family with a genetic disease. If chromosome A in a family also carried a disease-producing allele of a gene in this region and chromosome B carried a normal allele, finding a 1.2 kilobase (kb) band would indicate that the disease-producing allele was also present (both characteristics of chromosome A). Conversely, finding a 2.1-kb fragment would indicate that the normal allele of the gene was present (both characteristics of chromosome B). This type of genetic analysis is more fully discussed in the Medical Genetics section, Chapter 6.

A simple example is illustrated by the following case.

> A phenotypically normal man and woman have an 8-year-old son with sickle cell anemia. They also have a 5-year-old daughter who does not have sickle cell anemia but has not been tested for carrier status. The mother is in her 16th week of pregnancy and wishes to know whether the fetus that she is carrying will develop sickle cell disease. The mutation causing sickle cell anemia (G6V) also destroys a restriction site for the restriction endonuclease MstII. DNA from each of the family members is cut with MstII, Southern blotted, and probed with a ^{32}P-labeled cDNA probe for the 5′UTR of the β-globin gene. The results are shown below, along with the family pedigree. What is the best conclusion about the fetus?

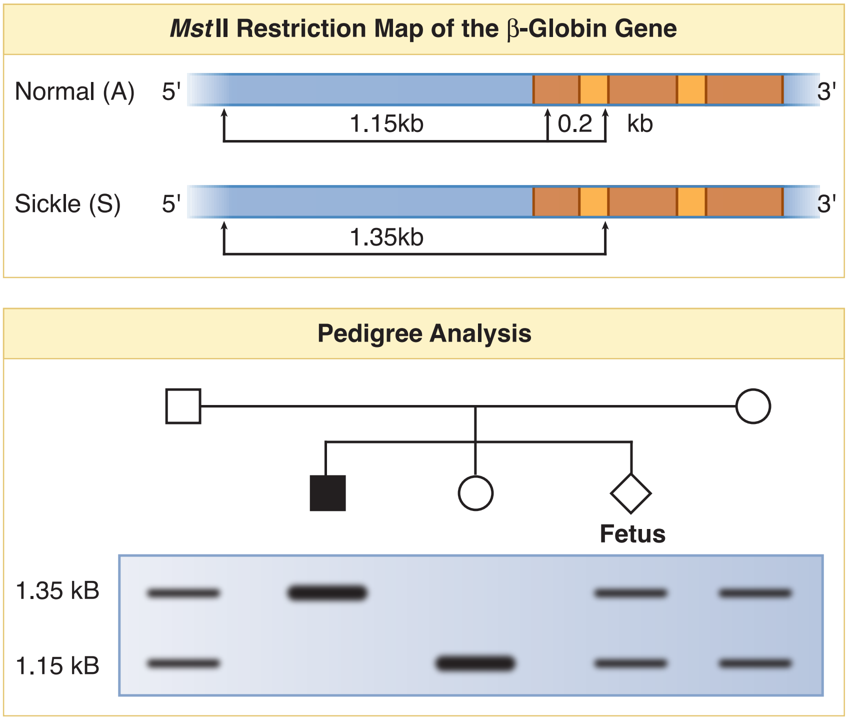


Figure I-7-3. RFLP Diagnosis of Sickle Cell Disease

(Answer: Fetus is heterozygous, or a carrier, for the mutation.)

Both the mother and the father have the same size restriction fragments marking the chromosome with the sickle allele. Because they are genetically unrelated, coming from different families this is not always the case. Additional examples will be discussed in Medical Genetics.

Northern Blots

High-Yield

Northern blots analyze RNA extracted from a tissue and are typically used to determine which genes are being expressed. One example is shown below. The goal is to determine which tissues express the *FMR1* gene involved in fragile X syndrome. RNA samples from multiple tissues have been separated by electrophoresis, blotted, and probed with a ^{32}P-cDNA probe from the *FMR1* gene. The results are consistent with high-level expression (a 4.4-kb transcript) of this gene in brain and testis and lower-level expression in the lung. In the heart, the gene is also expressed, but the transcripts are only 1.4 kb long. Variability in the lengths of the mRNAs transcribed from a single gene may be the result of alternative splicing as discussed in Chapter 3, Transcription and RNA Processing.

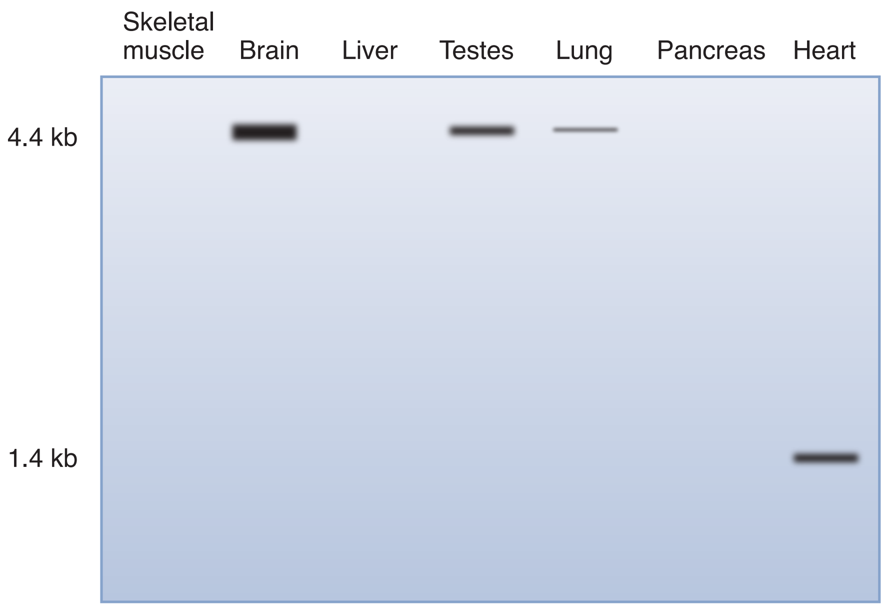


Figure I-7-4. Northern Blot to Determine Pattern of FMR1 Expression

Gene expression profiling (microarrays)

It is now possible to embed probes for many different mRNA in a multi-well gel or even on a chip to simultaneously determine whether hundreds of genes are expressed in a particular tissue. This is referred to as gene expression profiling or microarray analysis. For example, previous research has suggested that cells from a breast cancer express a variety of genes that are either not expressed or expressed only at a low level in normal cells. Probes for the corresponding mRNAs can be embedded on a solid support and total mRNA from a particular woman's breast tumor tested with each probe. The pattern of gene expression (gene expression profiling) may give information about the prognosis for that particular woman, aiding in making choices about the appropriate treatment protocol.

Clinical Correlate

Fragile X Syndrome

Fragile X syndrome is the leading known cause of inherited mental retardation. Other symptoms include large ears, elongated face, hypermobile joints, and macroorchidism in postpubertal males. The gene involved, *FMR1*, maps to the long arm of the X chromosome. See Section II, Chapter 1, for a further discussion of this single-gene disorder.

Western Blots

High-Yield

Western blots separate proteins by gel electrophoresis and use ^{125}I-labeled probe antibodies to detect the proteins (antigens). For this reason, Western blots are also referred to as immunoblots.

One important application of Western blotting is to detect the presence of antibodies to the HIV virus in HIV testing (*see* Immunology Lecture Notes). Western blots may also be used to identify whether a particular protein is in a cell and therefore represent a way to test for gene expression at the level of translation.

POLYMERASE CHAIN REACTION (PCR)

The polymerase chain reaction (PCR) is a technique in which a selected region of a chromosome can be amplified more than a million-fold within a few hours. The technique allows extremely small samples of DNA to be used for further testing. The PCR has many applications.

The region of a chromosome to be amplified by a PCR is referred to as the target sequence and may be an area containing a suspected mutation, a short tandem repeat (STR, or microsatellite sequence), or really any area of interest. The major constraint in performing a PCR is that one must know the nucleotide sequence bordering (flanking) the target region at each of its 3′ ends. This is no longer an obstacle because of the sequence data from the Human Genome Project.

The steps of the PCR include the following:

- Add the sample containing DNA to be amplified.
- Add excess amounts of primers complementary to both 3′ flanks of the target sequence. This selects the region to be amplified.
- Add a heat-stable DNA polymerase (*Taq* DNA polymerase) and deoxyribonucleotides (dNTPs) for DNA synthesis.
- Heat the sample to melt the DNA (convert dsDNA to ssDNA).
- Cool the sample to re-anneal the DNA. Because the ratio of primers to complementary strands is extremely high, primers bind at the 3′ flanking regions.
- Heat the sample to increase the activity of the *Taq* DNA polymerase. Primer elongation occurs, and new complementary strands are synthesized.

This process is repeated for approximately 20 cycles, producing over a million double-stranded copies of the target sequence.

Note

The PCR can be used for:

- Comparing DNA samples in forensic cases
- Paternity testing
- Direct mutation testing
- Diagnosing bacterial and viral infections
- HIV testing when antibody tests are uninformative (importantly, infants whose mothers are HIV- positive)

Note

Short tandem repeats (STRs, or microsatellites) are repeats of a di- to tetranucleotide sequence. They are useful in genetic testing.

- Occur both in the spacer regions between genes and within gene regions
- In noncoding regions, show some variability in length as mutations have expanded or contracted the number of repeats throughout evolution
- Have known positions that are documented in chromosome maps

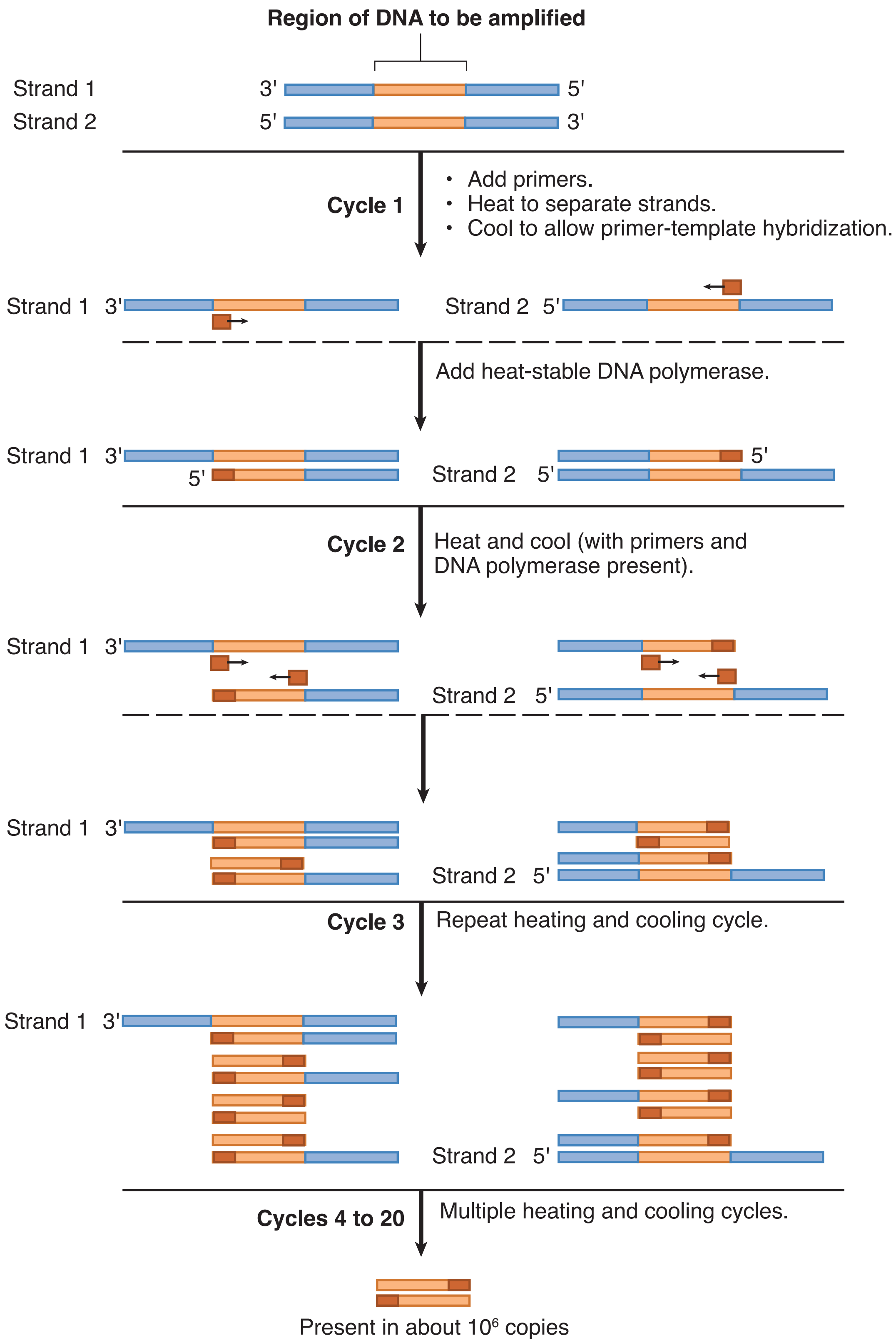


Figure I-7-5. Polymerase Chain Reaction

Agarose gel electrophoresis of PCR products

If the known mutation changes the length of the gene (e.g., microsatellite repeats), this difference can be detected in the PCR-amplified DNA by electrophoresis on agarose gel.

A 59-year-old man with increasing clumsiness, loss of balance, and irregular tremor and jerkiness in both arms seeks medical attention. The physician also notes impaired visual tracking. The patient has one sister, age 53. His father and mother died in a car accident at ages 45 and 43, respectively. There is no known history of neurologic dysfunction in his family. He takes a multiple vitamin tablet daily but no prescription drugs or supplements. MRI reveals visible atrophy of the caudate and total basal ganglia, and a tentative diagnosis of Huntington disease is made. To confirm the diagnosis, a sample of blood is sent for molecular genetic testing. PCR amplification is carried out on a region on 4p16.3 suspected to contain a mutation. The results are shown below, along with results from a normal, healthy, age-matched control. Is this result consistent with the diagnosis?

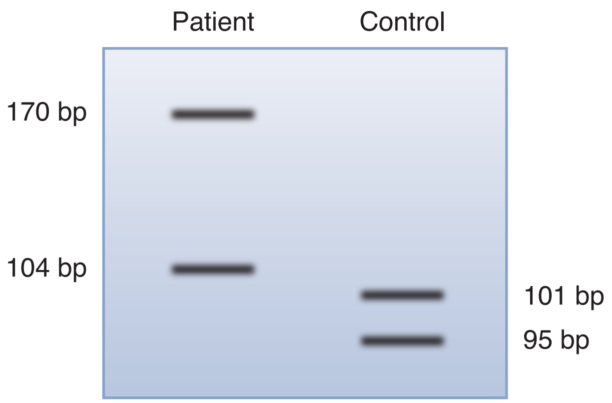


Figure I-7-6. Direct Genetic Diagnosis of Neurodegenerative Disease Using PCR

(Answer: Yes, the results are consistent with Huntington disease. In comparison with the normal control PCR products, one of the patient's PCR products (170 bp) is well out of the normal range. This is consistent with a triplet repeat expansion in that allele of the huntingtin gene. The 2 bands from the control are 95 base pairs and 101 base pairs, a difference of 2 triplet repeats. The patient sample shows a 104 base pair band, a difference of one triplet repeat from the larger control band. Significantly, there is a difference of 69 base pairs between the patient's 2 bands: 104 base pairs versus 170 base pairs. This is equivalent to 23 triplet repeats.)

Recall Question

Which of the following is an application of cDNA libraries rather than PCR technique?

A. Comparing DNA

B. Diagnosing specific viral infections

C. Producing transgenic animals

D. Paternity testing

Answer: C

Genetic Fingerprinting Using PCR Amplification of Microsatellite Sequences

High-Yield

Most repetitive sequences are not in coding regions. Because expansion of these sequences in spacer DNA rarely affects any function, they become highly polymorphic in the population and can be used to develop a genetic fingerprint. Such fingerprints are important in paternity testing and forensic medicine. Very small samples containing dried tissue can be analyzed by this technique. PCR amplification of repetitive sequences such as VNTR and STR sequences can be used for genetic fingerprinting.

Paternity testing using PCR amplification of microsatellite sequences

Although microsatellite sequences are distributed throughout the DNA, a single region may be selectively amplified by using primers that overlap the 3′-flanking regions adjacent to the repeat analyzed. Such primers amplify "single-locus" sequences, which are highly polymorphic within the population. Because humans have pairs of chromosomes, each individual will have a maximum of 2 bands, one from the father and one from the mother.

For instance, in the figure below, are the tested males in case 1 and case 2 the fathers of the children?

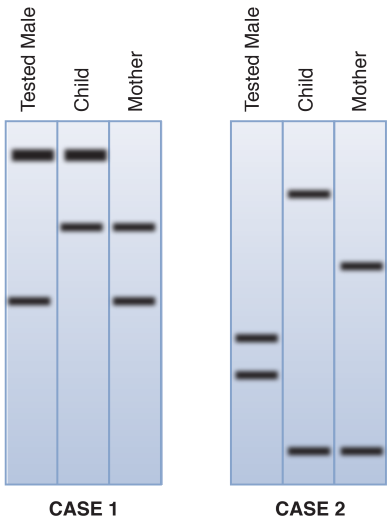


Figure I-7-7. Paternity Testing

The approach should be as follows:

- Identify the child's band in common with the mother. The other band must be from the father.
- See if the tested male has a band matching the band from the child. Draw a conclusion.

Case 1: The tested male in case 1 may be the father, as he shares a band with the child. We cannot be certain, however, because many other men in the population could have this same band. Matches are required at several different loci to indicate with high probability that a tested male is the father.

Case 2: The tested male in case 2 cannot be the father, as neither of his bands is shared with the child.

In practice, 9–10 different polymorphisms are necessary to indicate a match.

PCR in Direct Mutation Testing

If the mutation(s) causing a specific disease is known, the loci involved can be amplified with a PCR and further analyzed to determine whether the mutation(s) is present. Mutations causing a length difference can be detected by gel electrophoresis as a length difference in the PCR product. Mutations causing a sequence difference can be detected by testing the PCR products with ASOs.

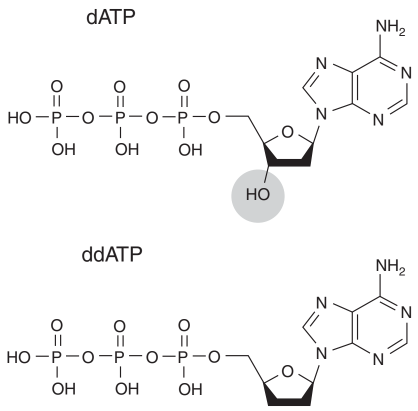
Sequencing DNA for direct mutation testing

If a mutation has been mapped to a particular region of a chromosome, one can use the PCR to amplify the region and sequence one of the 2 strands to determine whether it harbors a mutation. Double-stranded DNA is used along with many copies of a primer that binds only to one strand (often the coding strand).

- A sample of the DNA to be sequenced is put in each of 4 reaction mixtures containing a DNA polymerase and all the necessary deoxyribonucleotide triphosphates (dNTPs) required to synthesize new DNA.
- In each test tube a different dideoxynucleotide triphosphate (ddNTP) which lack both the 3' and 2' hydroxyl groups is added. The ddNTPs can be inserted into a growing chain of DNA, but then any subsequent elongation is stopped.
- The pieces of newly synthesized DNA in each tube are separated by gel electrophoresis in a different lane of the gel.
- The sequence of the new strand can be read from the smallest to the largest fragments on the gel, e.g., from the bottom to the top.

Note

Comparison of dATP and ddATP



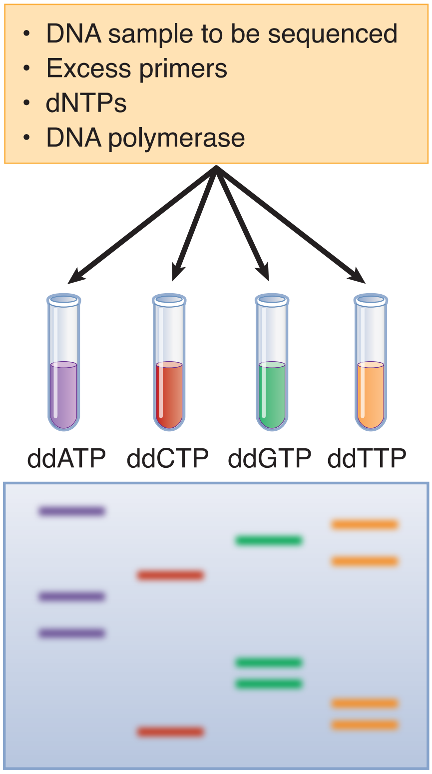


Figure I-7-8. DNA Sequencing

The sequence of the newly synthesized DNA strand in this example is 5'CTTG-GAACTGTA 3'.

If one wants the sequence of the original strand, serving as the template in the sequencing procedure, it would be complementary and anti-parallel to the sequence read from the gel. In this example the original strand sequence would be 5'TACAGTTCCAAG 3'.

PCR in HIV Testing

The enzyme-linked immunosorbent assay (ELISA) is used to screen individuals for antibodies to the HIV virus. The test has high sensitivity but somewhat lower specificity. A positive result in an ELISA must be confirmed by a Western blot for antibodies that are reactive with specific HIV protein antigens (*see* Immunology Lecture Notes).

In certain instances, ELISA/Western blot is not useful and PCR is the test of choice to detect HIV infection.

- PCR is designed to test for the integrated proviral genome, not for antibodies to HIV protein antigens.
- Primers that are specific for the HIV provirus are used for the PCR.
- If the person is infected, the proviral genome will be amplified and detected. If the person is not infected, there will be no PCR product detected.

A PCR for the HIV provirus has 2 important advantages over the ELISA/Western blot:

- Is positive much earlier after infection
- Does not rely on an antibody response by the individual

Important situations in which the PCR is currently used include **HIV testing in newborns whose mothers are HIV positive** (will always be positive in ELISA/Western blot) and **early testing after known exposure to HIV-positive blood** (e.g., needlesticks) or other fluids/tissue.

Clinical Correlate

In chronic myelogenous leukemia (CML), the presence of the Philadelphia chromosome translocation (t[9;22]) produces a BCR-ABL, an abnormal fusion protein with tyrosine kinase activity. It has been shown that monitoring the level of BCR-ABL mRNA with an RT-PCR in CML patients during therapy with Imatinib (a tyrosine kinase inhibitor) is helpful for both prognosis and management of therapy.

Reverse Transcriptase PCR (RT-PCR)

An RT-PCR detects and can quantify a specific RNA rather than DNA in a sample. This test is useful in detecting RNA viruses such as HIV and, in situations similar to the Northern blot, determining whether a gene is transcribed.

Measuring viral load in AIDS patients

The RT-PCR is used to measure the concentration of active circulating virus in the blood of an AIDS patient (viral load). In this way, the test can be used to monitor the status of infection and the infection's response to antiviral drugs.

Figure I-7-9A shows the steps involved. A blood sample from an HIV-infected individual is obtained and, after appropriate preparation, is treated with reverse transcriptase to produce cDNA from any RNA in the sample. The cDNA is subsequently PCR-amplified using primers specific for the end sequences of the HIV cDNA. The amplified product is quantitated and, with the use of a standard curve (Figure I-7-9B), can be related to the original amount of HIV RNA present.

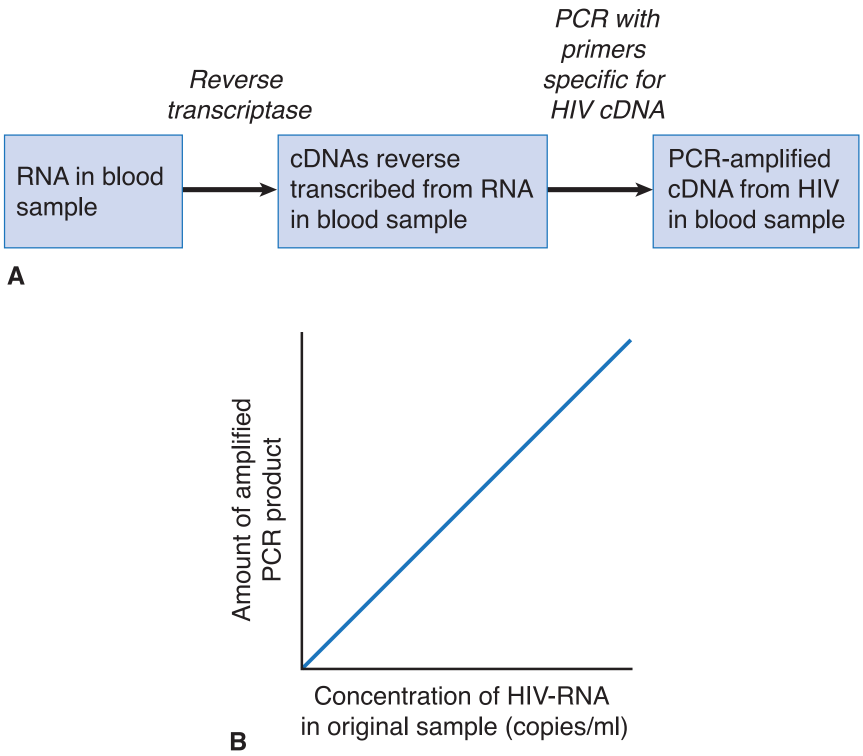
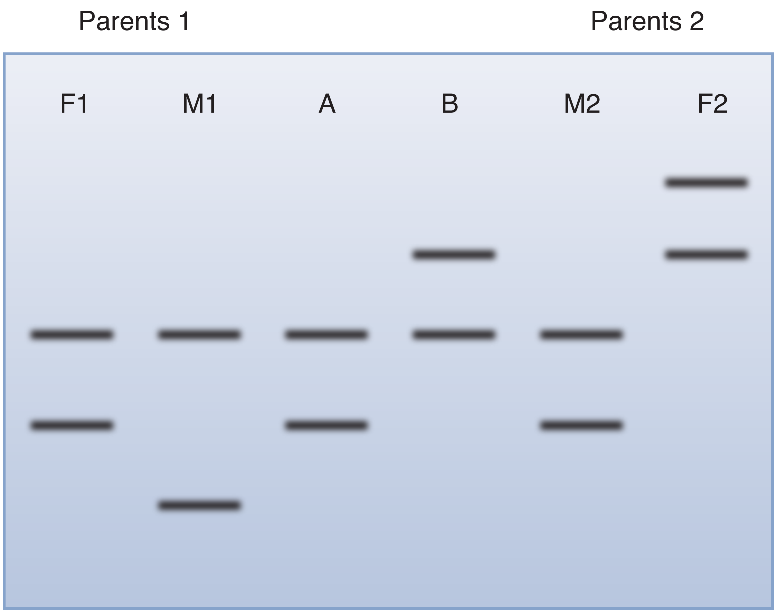


Figure I-7-9. Quantifying Viral Load in HIV Infection Using a Reverse Transcriptase PCR (RT-PCR)
(A) RT-PCR technique (B) Standard curve for quantifying HIV-RNA in blood sample

Review Questions

1. Two sets of parents were friends in a small town and had babies on the same day. The wristbands of the two similar-looking infants (A and B) were inadvertently mixed at the pediatric care unit. In order to accurately identify the parents of the respective infants, PCR analysis was performed on samples of blood taken from the two infants and both sets of parents (Father 1 and Mother 1 versus Father 2 and Mother 2). Shown below is the analysis of the PCR products by gel electrophoresis.



What is the best conclusion from the analysis?

A. A is the child of Parents 1.
B. A is the child of Parents 2.
C. B is the child of Parents 1.
D. Father 1 (F1) could be the father of both infants.
E. (Father 2 (F2) could be the father of both infants.

2. Paternal relationship between a man and infant can be best determined by the technique commonly referred to as DNA fingerprinting. Which of the following sequences is most conveniently analyzed in a DNA fingerprint?

A. Histocompatibility loci
B. Centromeres
C. Microsatellite tandem repeats (STRs)
D. Restriction enzyme sites
E. (Single-copy sequences

3. Sickle cell anemia is caused by a missense mutation in codon 6 of the β-globin gene.

	Codon number 5 6 7 8
Normal allele	CCT G**A**G GAG AAG
Mutant allele	CCT G**T**G GAG AAG

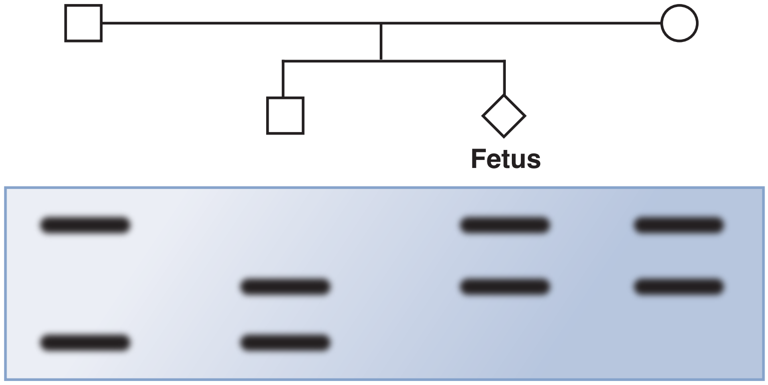
A man with sickle cell disease and his phenotypically normal wife request genetic testing because they are concerned about the risk for their unborn child. DNA samples from the man and the woman and from fetal cells obtained by amniocentesis are analyzed using the PCR to amplify exon 1 of the β-globin gene. Which 12-base nucleotide sequence was most likely used as a specific probe complementary to the coding strand of the sickle cell allele?

A. CCTCACCTCAGG
B. CCTGTGGAGAAG
C. GGACACCTCTTC
D. CTTCTCCACAGG
E. CTTCTCCTCAGG

4. mRNA encoding glucose 6-phosphatase was isolated from baboon liver and used to make a ^{32}P-cDNA probe. DNA was then isolated from marmoset and from human tissue, digested with a restriction endonuclease, Southern blotted, and probed with the ^{32}P-cDNA. Which of the following conclusions can be drawn from the results of this analysis shown below?

A. The glucose 6-phosphatase gene is present in baboon, marmoset and human liver.
B. Both marmoset and human liver express the glucose 6-phosphatase gene.
C. There are two glucose 6-phosphatase genes in the human liver.
D. The glucose 6-phosphatase gene is on different chromosomes in the marmoset and in the human.
E. The human and marmoset tissue used in this experiment is from liver.

5. A couple seeks genetic counseling because both the man and the woman (unrelated to each other) are carriers of a mutation causing β-thalassemia, an autosomal recessive condition. The couple has one son who is phenotypically normal and has been shown by DNA analysis to be homozygous for the normal allele. They wish to know whether the fetus in the current pregnancy will have β-thalassemia. Using a probe for the β-globin gene that detects a *Bam*HI RFLP, the following results are obtained. What is the best conclusion about the fetus?



A. The fetus has inherited the mutation from both parents.
B. The fetus has inherited the mutation from the mother but not from the father.
C. The fetus has inherited the mutation from the father but not from the mother.
D. The fetus has not inherited the mutation from either parent.
E. The results are inconclusive.

Answers

1. **Answer: A.** Among the conclusions offered, only A is consistent with the results on the blot. Infant A's pattern shows a PCR product (lower on the blot) matching F1 and another PCR product (higher on the blot) matching M1. Neither of infant A's PCR products match F2 **(choices B and E).** The upper PCR product in infant B's pattern does not match with either F1 or M1 **(choices C and D).** Although unlikely given the situation, another possibility is consistent with the blot. Infant A could be the child of M2 and F1, although this is not offered as an option.

2. **Answer: C.** STR sequences are amplified using a PCR and analyzed by gel electrophoresis. Although RFLP analysis could potentially be used for this purpose, it is not the method of choice.

3. **Answer: D.** The complementary probe will be antiparallel to the coding strand of the mutant allele, with all sequences written $5' \rightarrow 3'$.

4. **Answer: A.** All 3 tissues contain the gene (the probe was produced from baboon mRNA, implying the gene is also there).

5. **Answer: C.** Knowing the son is homozygous for the normal allele, one can conclude that the two restriction fragments shown in his pattern derived from chromosomes without the mutation. It is also clear that the upper (larger) fragment came from his mother's chromosome and the lower (smaller) fragment came from his father's chromosome. The fetus has the fragment from his mother's normal chromosome. The other fragment (top one on the blot) must have come from the father's chromosome with the mutation. The fetus therefore is heterozygous for the mutation and the normal allele of the β-globin gene.

Amino Acids, Proteins, and Enzymes

8

Learning Objectives

- ❑ Explain information related to amino acids
- ❑ Answer questions about protein turnover and amino acid nutrition
- ❑ Understand fundamental concepts of enzyme kinetics

AMINO ACIDS

General Structure

All amino acids have a central carbon atom attached to a carboxyl group, an amino group, and a hydrogen atom. The amino acids differ from one another only in the chemical nature of the side chain (R).

There are hundreds of amino acids in nature, but only 20 are used as building blocks of proteins in humans.

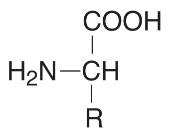


Figure I-8-1. Generalized Structure of Amino Acids

Classification

High-Yield

The amino acids can be classified as hydrophobic or hydrophilic, depending on the ease with which their side chains interact with water. In general, proteins fold so that amino acids with hydrophobic side chains are in the interior of the molecule where they are protected from water, while those with hydrophilic side chains are on the surface.

Hydrophobic amino acids

- Phenylalanine and tyrosine are precursors for catecholamines.
- Tryptophan can form serotonin and niacin.
- Valine, leucine, and isoleucine are branched-chain amino acids whose metabolism is abnormal in maple syrup urine disease (see chapter 17).
- Proline is a secondary amine whose presence in a protein disrupts normal secondary structure.

Hydrophilic amino acids

- Have side chains that contain O or N atoms; some of the hydrophilic side chains are charged at physiologic pH
- The acidic amino acids (aspartic and glutamic acids) have carboxyl groups that are negatively charged, whereas the basic amino acids (lysine, arginine, and histidine) have nitrogen atoms that are positively charged.

Additional points about some of these amino acids include:

- Serine and threonine are sites for O-linked glycosylation of proteins, a posttranslational modification that should be associated with the Golgi apparatus.
- Asparagine is a site for N-linked glycosylation of proteins, a cotranslational modification that should be associated with the endoplasmic reticulum.
- Cysteine contains sulfur and can form disulfide bonds to stabilize the shape (tertiary structure) of proteins. Destroying disulfide bonds denatures proteins.

- Methionine, another sulfur-containing amino acid, is part of S-adenosylmethionine (SAM), a methyl donor in biochemical pathways.

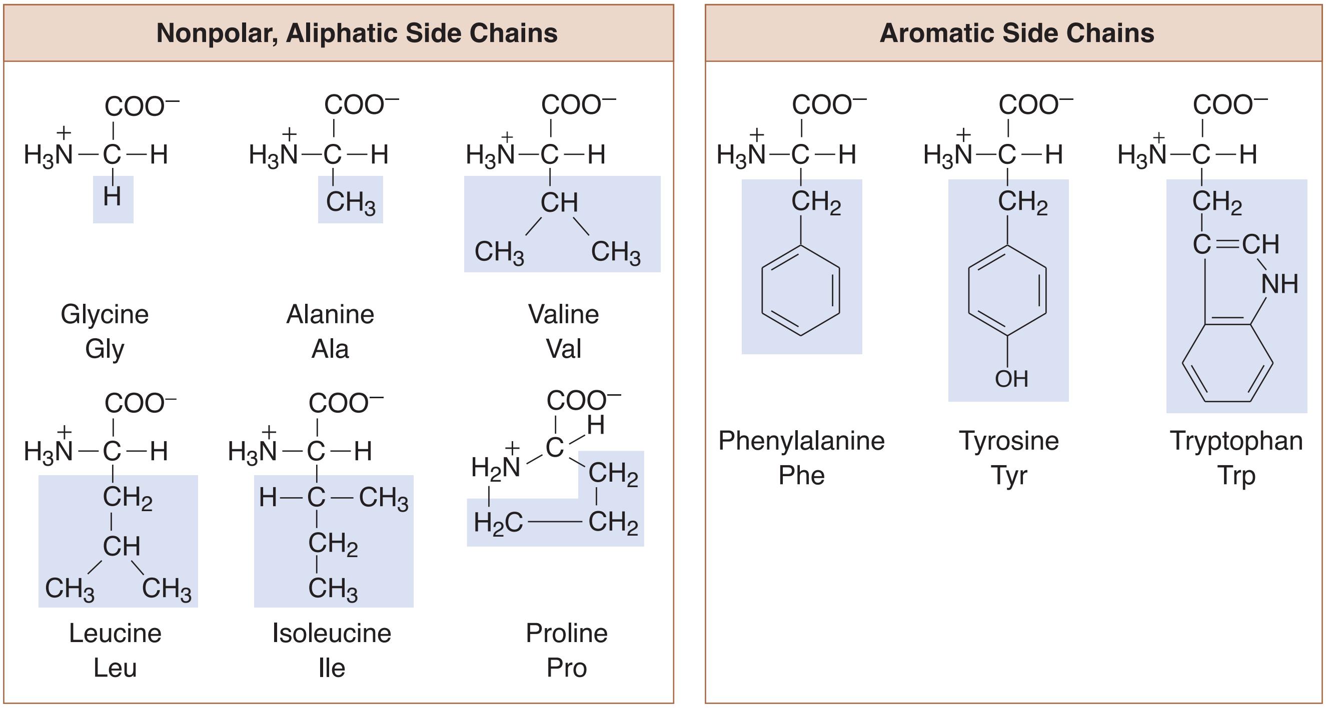


Figure I-8-2. The Hydrophobic Amino Acids

Note: Tyrosine can be considered nonpolar or polar because of the ability of the -OH group to form a hydrogen bond.

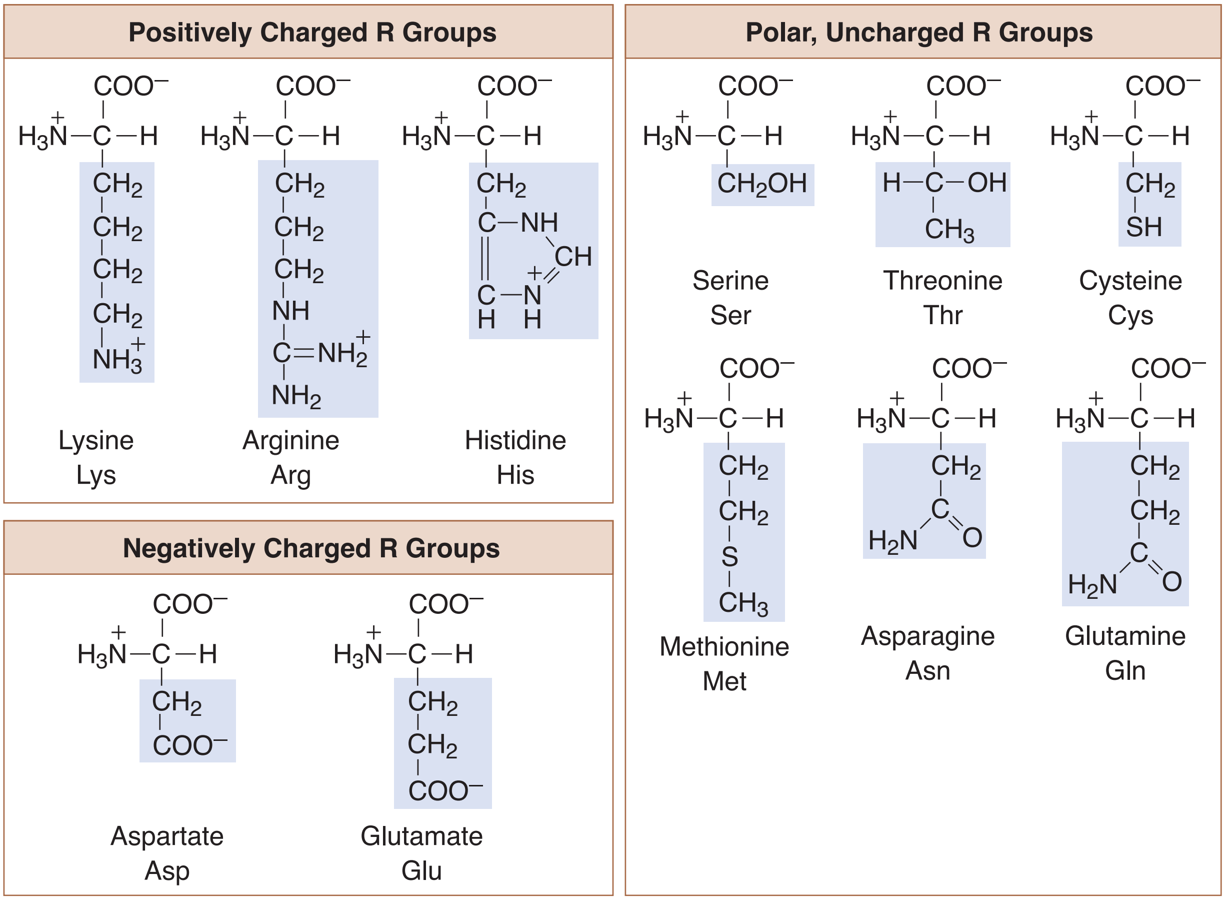
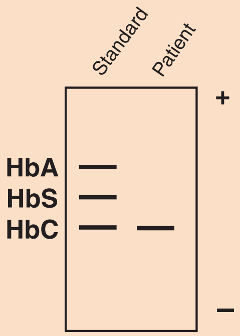


Figure I-8-3. The Hydrophilic Amino Acids

Note: Methionine can be considered nonpolar or polar because it contains a sulfur.

Hemoglobinopathy

An 8-year-old African American boy was experiencing pain in the chest and back. He was taken to the hospital, where he was found to have mild anemia, splenomegaly, and rod-shaped crystals in the erythrocytes. A preliminary diagnosis of sickle cell anemia was made. To validate the diagnosis, a small aliquot of his blood was subjected to electrophoresis to determine the identity of the hemoglobin in his erythrocytes. After reviewing the data, the physician concluded that he did not have sickle cell anemia, but rather a sickle cell anemia–like hemoglobinopathy with the relatively common mutation of HbC.



Sickle cell anemia is characterized by severe pain in the bones, abdomen, and chest, along with periods of hemolytic problems. Episodes of vaso-occlusive pain lasting approximately 1 week are a frequent problem. These crises are often precipitated by dehydration or infection. A widely used method to analyze hemoglobins found in various hemoglobinopathies is electrophoresis at pH 8.4, where single amino acid substitutions can be easily detected. In sickle cell anemia, there is a substitution of valine for glutamate at position 6 in Hb, meaning that the HbS will have one less negative charge overall compared with HbA. In HbC, there is a substitution of lysine for glutamate at position 6, meaning that HbC will have two additional positive charges compared with HbA. These 3 hemoglobins can be resolved by electrophoresis.

PROTEIN TURNOVER AND AMINO ACID NUTRITION

When older proteins are broken down in the body, they must be replaced. This concept is called protein turnover, and different types of proteins have very different turnover rates. Protein synthesis occurs during the process of translation on ribosomes. Protein breakdown tends to occurs in 2 cellular locations:

- Lysosomal proteases digest endocytosed proteins.
- Large cytoplasmic complexes (or proteasomes) digest older or abnormal proteins that have been covalently tagged with a protein (called *ubiquitin*) for destruction.

Essential Amino Acids

All 20 types of amino acids are required for protein synthesis. These amino acids can be derived from digesting dietary protein and absorbing their constituent amino acids or, alternatively, by synthesizing them *de novo*.

There are 10 amino acids that cannot be synthesized in humans and thus must be provided from dietary sources. These are called the **essential amino acids**.

Arginine is required only during periods of growth or positive nitrogen balance.

Table I-8-1. Essential Amino Acids

Arginine*	Methionine
Histidine	Phenylalanine
Isoleucine	Threonine
Leucine	Tryptophan
Lysine	Valine

*Essential only during periods of positive nitrogen balance.

Nitrogen Balance

Nitrogen balance is the (normal) condition in which the amount of nitrogen incorporated into the body each day exactly equals the amount excreted.

Negative nitrogen balance occurs when nitrogen loss exceeds incorporation. It is associated with:

- Protein malnutrition (kwashiorkor)
- Dietary deficiency of even 1 essential amino acid
- Starvation
- Uncontrolled diabetes
- Infection

Positive nitrogen balance occurs when the amount of nitrogen incorporated exceeds the amount excreted. It is associated with:

- Growth
- Pregnancy
- Convalescence (recovery phase of injury or surgery)
- Recovery from condition associated with negative nitrogen balance

Note

Do not confuse kwashiorkor with marasmus. **Marasmus** is a chronic deficiency of calories; patients do not present with edema as they do with kwashiorkor.

Note

Hydrolysis of high-energy bonds in ATP or GTP provide energy to drive reactions in which ΔG >0.

BIOCHEMICAL REACTIONS

Chemical reactions have 2 independent properties: energy and rate.

ΔG represents the amount of energy released or required per mole of reactant. The amount or sign of ΔG indicates nothing about the rate of the reaction.

Table I-8-2. Energy versus Rate

Energy (ΔG)	Rate (v)
Not affected by enzymes	Increased by enzymes
ΔG <0, thermodynamically spontaneous (energy released, often irreversible)	Decrease energy of activation, $\Delta G^{\ddagger}$
ΔG >0, thermodynamically nonspontaneous (energy required)	
ΔG = 0, reaction at equilibrium (freely reversible)	
ΔG^0 = energy involved under standardized conditions	

The rate of the reaction is determined by the energy of activation ($\Delta G^{\ddagger}$), which is the energy required to initiate the reaction. ΔG and $\Delta G^{\ddagger}$ are represented in the figure below. Enzymes lower the energy of activation for a reaction; they do not affect the value of ΔG or the equilibrium constant for the reaction, K_{eq}.

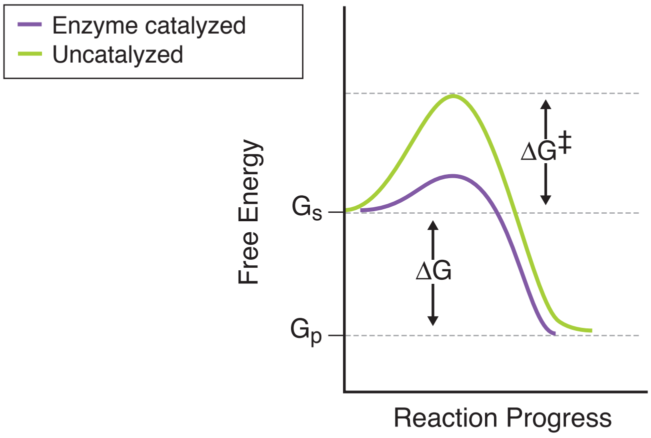


Figure I-8-4. Energy Profile for a Catalyzed and Uncatalyzed Reaction

Michaelis-Menten Equation

The Michaelis-Menten equation describes how the rate of the reaction, V, depends on the concentration of both the enzyme [E] and the substrate [S], which forms product [P].

$$E + S \rightleftharpoons E-S \rightarrow E + P$$

$$V = \frac{k_2[E][S]}{K_m + [S]} \text{ or, with [E] held constant, } V = \frac{V_{max}[S]}{K_m + [S]}$$

Note: $V_{max} = k_2[E]$

V_{max} is the maximum rate possible to achieve with a given amount of enzyme. The only way to increase V_{max} is by increasing the [E]. In the cell, this can be accomplished by inducing the expression of the gene encoding the enzyme.

The other constant in the equation, K_m is often used to compare enzymes. K_m is the substrate concentration required to produce half the maximum velocity. Under certain conditions, K_m is a measure of the affinity of the enzyme for its substrate. When comparing two enzymes, the one with the higher K_m has a lower affinity for its substrate. The K_m value is an intrinsic property of the enzyme-substrate system and cannot be altered by changing [S] or [E].

When the relationship between [S] and V is determined in the presence of constant enzyme, many enzymes yield the graph shown below, a hyperbola.

Bridge to Medical Genetics

A missense mutation in the coding region of a gene may yield an enzyme with a different K_m.

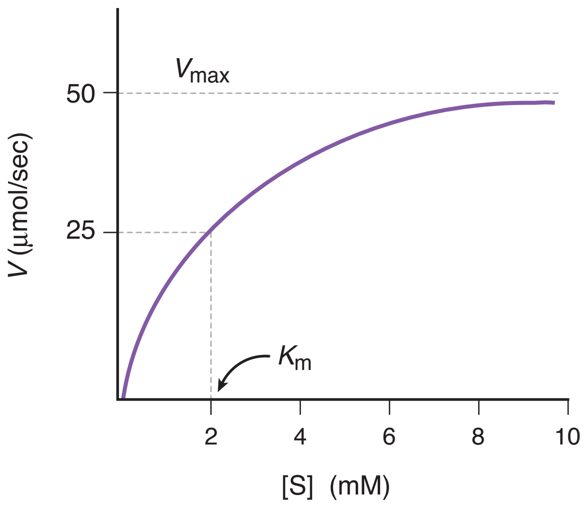


Figure I-8-5. Michaelis-Menten Plot

Recall Question

Which of the following conditions will result in arginine becoming an essential amino acid?

A. Diabetes

B. Pregnancy

C. Sepsis

D. Starvation

Answer: B

Lineweaver-Burk Equation

The Lineweaver-Burk equation is a reciprocal form of the Michaelis-Menten equation. The same data graphed yield a straight line, as shown below.

The actual data are represented by the portion of the graph to the right of the *y*-axis, but the line is extrapolated into the left quadrant to determine its intercept with the *x*-axis. The intercept of the line with the *x*-axis gives the value of $-1/K_m$. The intercept of the line with the *y*-axis gives the value of $1/V_{max}$.

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

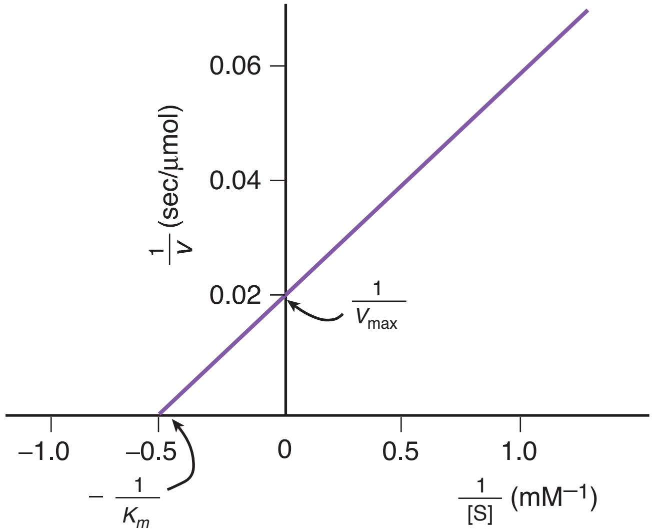


Figure I-8-6. Lineweaver-Burk Plot

Inhibitors and Activators

Competitive inhibitors resemble the substrate and compete for binding to the active site of the enzyme. **Noncompetitive inhibitors** do not bind at the active site; they bind to regulatory sites on the enzyme.

Table I-8-3. Important Classes of Enzyme Inhibitors

Class of Inhibitor	K_m	V_{max}
Competitive	Increase	No effect
Noncompetitive	No effect	Decrease

The effects of these classes of inhibitors on Lineweaver-Burk kinetics are shown below. Notice that on a Lineweaver-Burk graph, inhibitors always lie above the control on the right side of the *y*-axis.

Note

Many drugs are **competitive inhibitors** of key enzymes in pathways.

- The statin drugs (lovastatin, simvastatin), used to control blood cholesterol, competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase in cholesterol biosynthesis.
- Methotrexate, an antineoplastic drug, competitively inhibits dihydrofolate reductase, depriving the cell of active folate needed for purine and deoxythymidine synthesis, thus interfering with DNA replication during S phase.

An example of a **noncompetitive inhibitor** is allopurinol, which noncompetitively inhibits xanthine oxidase.

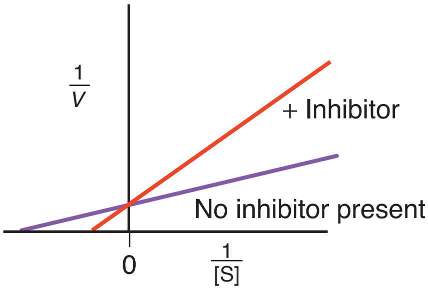


Figure I-8-7. Lineweaver-Burk Plot of Competitive Inhibition

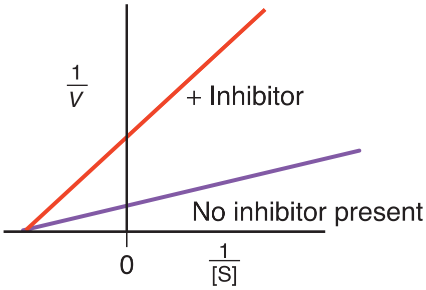


Figure I-8-8. Lineweaver-Burk Plot of Noncompetitive Inhibition

Figure I-8-9 shows the effect on a Lineweaver-Burk plot of adding more enzyme. It might also represent adding an activator to the existing enzyme or a covalent modification of the enzyme. An enzyme activator is a molecule that binds to an enzyme and increases its activity. In these latter two cases the K_m might decrease and/or the V_{max} might increase but the curve would always be below the control curve in the right-hand quadrant of the graph.

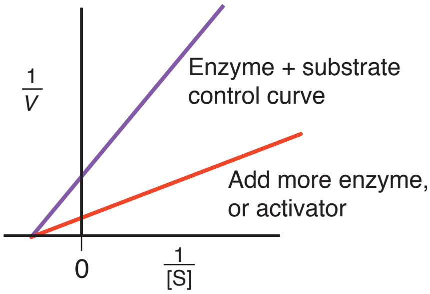


Figure I-8-9. Lineweaver-Burk Plot Showing the Addition of More Enzyme or the Addition of an Activator

Cooperative Enzyme Kinetics

High-Yield

Certain enzymes do not show the normal hyperbola when graphed on a Michaelis-Menten plot ([S] versus *V*), but rather show sigmoid kinetics owing to cooperativity among substrate binding sites. Cooperative enzymes have multiple subunits and multiple active sites. Enzymes showing cooperative kinetics are often regulatory enzymes in pathways (for example, phosphofructokinase-1 [PFK-1] in glycolysis).

In addition to their active sites, these enzymes often have multiple sites for a variety of activators and inhibitors (e.g., AMP, ATP, citrate, fructose-2,6-bisphosphate [F2,6-BP]). Cooperative enzymes are sometimes referred to as allosteric enzymes because of the shape changes that are induced or stabilized by binding substrates, inhibitors, and activators.

Bridge to Pharmacology

Methanol poisoning (wood alcohol poisoning) is treated with ethanol administration. Both are substrates for alcohol dehydrogenase (ADH), with ethanol having a much lower K_m for the enzyme compared with methanol. This prevents methanol from being converted to formaldehyde, which is toxic and not metabolized further.

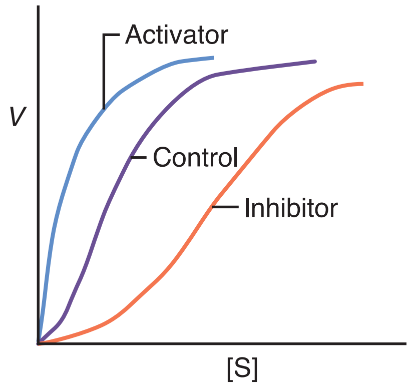


Figure I-8-10. Cooperative Kinetics

Transport Kinetics

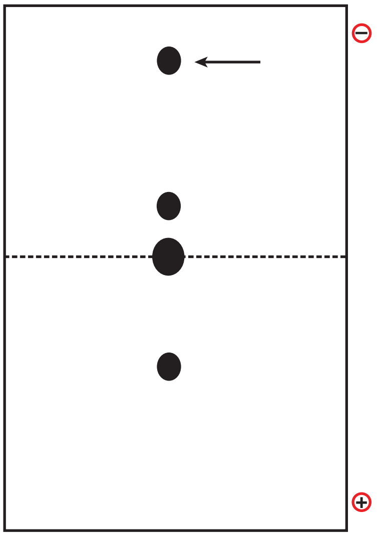
High-Yield

The K_m and V_{max} parameters that apply to enzymes are also applicable to transporters in membranes. The kinetics of transport can be derived from the Michaelis-Menten and Lineweaver-Burk equations, where K_m refers to the solute concentration at which the transporter is functioning at half its maximum activity. The importance of K_m values for membrane transporters is exemplified with the variety of glucose transporters (GLUT) and their respective physiologic roles (*see* Chapter 12).

Review Questions

Select the ONE best answer.

1. The peptide ala-arg-his-gly-glu is treated with peptidases to release all of the amino acids. The solution is adjusted to pH 7, and electrophoresis is performed. In the electrophoretogram depicted below, the amino acid indicated by the arrow is most likely to be



   A. glycine
   B. arginine
   C. glutamate
   D. histidine
   E. alanine

2. The reaction catalyzed by hepatic phosphofructokinase-1 has a ΔG^0 value of –3.5 kcal/mol. This value indicates that under standard conditions this reaction

   A. is reversible
   B. occurs very slowly
   C. produces an activator of pyruvate kinase
   D. is inhibited by ATP
   E. has a low energy of activation
   F. will decrease in activity as the pH decreases
   G. cannot be used for gluconeogenesis
   H. shows cooperative substrate binding
   I. is indirectly inhibited by glucagon
   J. is stimulated by fructose 2,6-bisphosphate

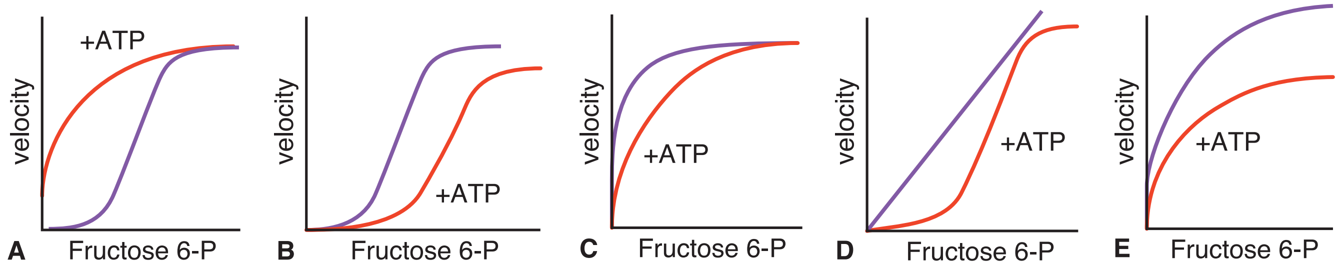
3. The activity of an enzyme is measured at several different substrate concentrations, and the data are shown in the table below.

[S] (mM)	V_0 (mmol/sec)
0.010	2.0
0.050	9.1
0.100	17
0.500	50
1.00	67
5.00	91
10.0	95
50.0	99
100.0	100

K_m for this enzyme is approximately

A. 50.0
B. 10.0
C. 5.0
D. 1.0
E. 0.5

4. Which of the diagrams illustrated below best represents the effect of ATP on hepatic phosphofructokinase-1 (PFK-1)?



5. Several complexes in the mitochondrial electron transport chain contain non-heme iron. The iron in these complexes is bound tightly to the thiol group of which amino acid?

   A. Glutamine
   B. Methionine
   C. Cysteine
   D. Tyrosine
   E. Serine

Items 6–8

Consider a reaction that can be catalyzed by one of two enzymes, A and B, with the following kinetics.

	K_m (M)	*V*max (mmol/min)
A.	5×10^{-6}	20
B.	5×10^{-4}	30

6. At a concentration of 5×10^{-6} M substrate, the velocity of the reaction catalyzed by enzyme A will be

   A. 10
   B. 15
   C. 20
   D. 25
   E. 30

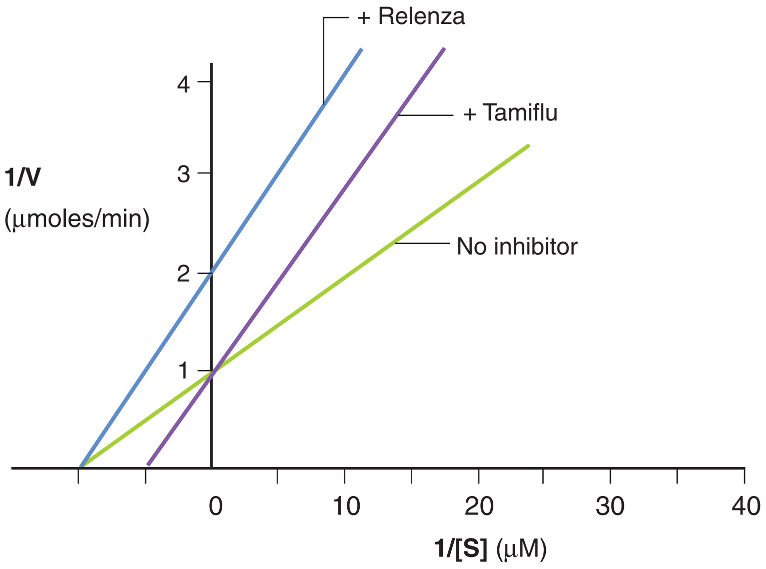
7. At a concentration of 5×10^{-4} M substrate, the velocity of the reaction catalyzed by enzyme B will be

   A. 10
   B. 15
   C. 20
   D. 25
   E. 30

8. At a concentration of 5×10^{-4} M substrate, the velocity of the reaction catalyzed by enzyme A will be

   A. 10
   B. 15
   C. 20
   D. 25
   E. 30

9. A worldwide pandemic of influenza caused by human-adapted strains of avian influenza or bird flu is a serious health concern. One drug for treatment of influenza, Tamiflu (oseltamivir), is an inhibitor of the influenza viral neuraminidase required for release of the mature virus particle from the cell surface. Recent reports have raised concerns regarding viral resistance of Tamiflu compelling the search for alternative inhibitors. Another drug, Relenza (zanamavir), is already FDA approved for use in a prophylactic nasal spray form. The graph below show kinetic data obtained for viral neuraminidase activity (measured as the release of sialic acid from a model substrate) as a function of substrate concentration in the presence and absence of Relenza and Tamiflu.



Based on the kinetic data, which of the following statements is correct?

A. Both drugs are competitive inhibitors of the viral neuraminidase.

B. Both drugs are noncompetitive inhibitors of the viral neuraminidase.

C. Tamiflu increases the K_m value for the substrate compared to Relenza

D. Relenza increases the V_{max} value for the substrate compared to Tamiflu.

E. Relenza is not an inhibitor of neuraminidase, but inhibits another viral enzyme.

Answers

1. **Answer: B.** Arginine is the most basic of the amino acids (pI~11) and would have the largest positive charge at pH 7.

2. **Answer: G.** The negative ΔG^0 value indicates the reaction is thermodynamically favorable (irreversible), requiring a different bypass reaction for conversion of F1, 6BP to F6P in the gluconeogenic pathway.

3. **Answer: E.** Because the apparent V_{max} is near 100 mmol/sec, $V_{max}/2$ equals 50 mmol/sec. The substrate concentration giving this rate is 0.50 mM.

4. **Answer: B.** Sigmoidal control curve with ATP inhibiting and shifting curve to the right is needed.

5. **Answer: C.** Cysteine has a sulfhydryl group in its side chain. Although methionine has a sulfur in its side chain, a methyl group is attached to it.

6. **Answer: A.** At the concentration of 5×10^{-6} M, enzyme A is working at one-half of its V_{max} because the concentration is equal to the K_m for the substrate. Therefore, one-half of 20 mmol/min is 10 mmol/min.

7. **Answer: B.** At the concentration of 5×10^{-4} M, enzyme B is working at one-half of its V_{max} because the concentration is equal to the K_m for the substrate. Therefore, one-half of 30 mmol/min is 15 mmol/min.

8. **Answer: C.** At the concentration of 5×10^{-4} M, 100 × the substrate concentration at K_m, enzyme A is working at its V_{max}, which is 20 mmol/min.

9. **Answer: B.** Based on the graph, when the substrate is present, Tamiflu results in the same V_{max} and higher K_m compared to the line when no inhibitor added. These are hallmarks of competitive inhibitors of enzymes, which Tamiflu is. Noncompetitive inhibitors result in decreased V_{max} and the same K_m with no inhibitor added, which is shown by the Relenza line in the graph.

Hormones 9

Learning Objectives

- ❑ Understand concepts concerning hormones and signal transduction
- ❑ Interpret scenarios about mechanism of water-soluble hormones
- ❑ Answer questions about G-proteins and second messengers in signal transduction

HORMONES AND SIGNAL TRANSDUCTION

Broadly speaking, a hormone is any compound produced by a cell, which by binding to its cognate receptor alters the metabolism of the cell bearing the hormone–receptor complex. Although a few hormones bind to receptors on the cell that produces them (autoregulation or autocrine function), hormones are more commonly thought of as acting on some other cell, either close by (paracrine) or at a distant site (telecrine).

- **Paracrine hormones** are secreted into the interstitial space and generally have a very short half-life. These include the prostaglandins and the neurotransmitters.
- **Telecrine hormones** are secreted into the bloodstream, generally have a longer half-life, and include the endocrine and gastrointestinal (GI) hormones. (The endocrine hormones are the classic ones, and it is sometimes implied that reference is being made to endocrine hormones when the word *hormones* is used in a general sense.)

Hormones are divided into 2 categories: those that are water soluble (hydrophilic) and those that are lipid soluble (lipophilic, or hydrophobic).

Note

The GI and endocrine hormones are discussed in detail in the GI and endocrinology chapters in the Physiology Lecture Notes. Although there is some overlap, this chapter presents basic mechanistic concepts applicable to all hormones, whereas coverage in the Physiology Notes emphasizes the physiologic consequences of hormonal action.

Table I-9-1. Two Classes of Hormones

Water-Soluble	Lipid-Soluble
Receptor in cell membrane	Receptor inside cell
Second messengers often involved Protein kinases activated	Hormone–receptor complex binds hormone response elements (HRE) of enhancer regions in DNA
Protein phosphorylation to modify activity of enzymes (requires minutes)	—
Control of gene expression through proteins such as cAMP response element binding (CREB) protein (requires hours)	Control of gene expression (requires hours)
Examples: • Insulin • Glucagon • Catecholamines	Examples: • Steroids • Calcitriol • Thyroxines • Retinoic acid

MECHANISM OF WATER-SOLUBLE HORMONES

Water-soluble hormones must transmit signals to affect metabolism and gene expression without themselves entering the cytoplasm. They often do so via second messenger systems that activate protein kinases.

Protein Kinases

High-Yield

A protein kinase is an enzyme that phosphorylates other proteins, changing their activity (e.g., phosphorylation of acetyl CoA carboxylase inhibits it). Examples of protein kinases are listed in Table I-9-2 along with the second messengers that activate them.

Table I-9-2. Signal Transduction by Water-Soluble Hormones

Pathway	G Protein	Enzyme	Second Messenger(s)	Protein Kinase	Examples
cAMP	G_s (G_i)	Adenyl cyclase	cAMP	Protein kinase A	Glucagon Epinephrine (β, α-2) Vasopressin (V2, ADH) kidney
PIP_2	G_q	Phospholipase C	DAG, IP_3, Ca^{2+}	Protein kinase C	Vasopressin (V1, V3) vascular smooth muscle Epinephrine (α_1)
cGMP	None	Guanyl cyclase	cGMP	Protein kinase G	Atrial natriuretic factor (ANF) Nitric oxide (NO)
Insulin, growth factors	Monomeric $p21^{ras}$	—	—	Tyrosine kinase activity of receptor	Insulin Insulin-like growth factor (IGF) Platelet-derived growth factor (PDGF) Epidermal growth factor (EGF)

Some water-soluble hormones bind to receptors with intrinsic protein kinase activity (often tyrosine kinases). In this case, no second messenger is required for protein kinase activation. The insulin receptor is an example of a tyrosine kinase receptor.

Activation of a protein kinase causes:

- Phosphorylation of enzymes to rapidly increase or decrease their activity.
- Phosphorylation of gene regulatory proteins such as CREB to control gene expression, usually over several hours. The typical result is to add more enzyme to the cell. CREB induces the phosphoenolpyruvate carboxykinase (PEPCK) gene. Kinetically, an increase in the number of enzymes means an increase in V_{max} for that reaction.

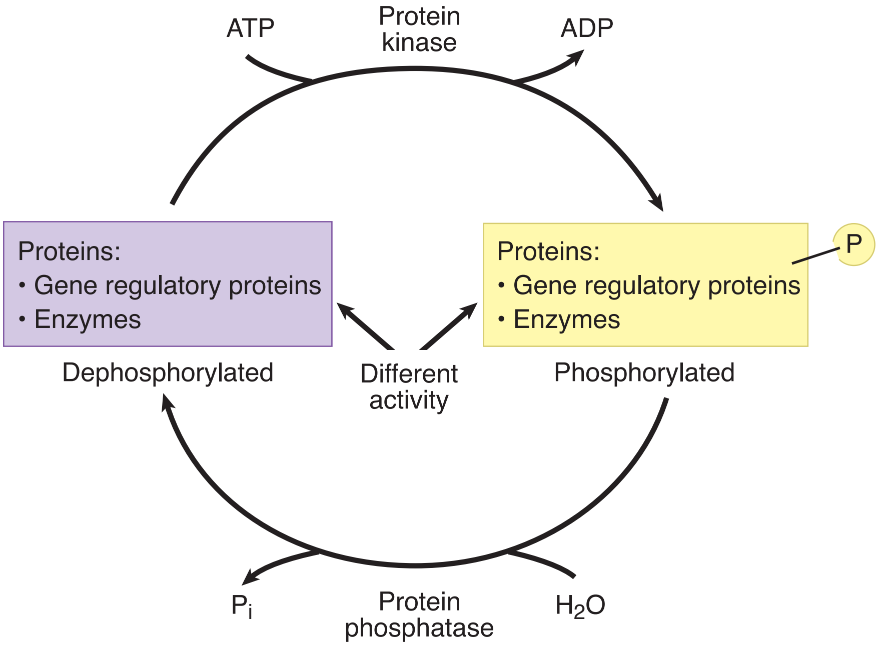


Figure I-9-1. Protein Kinases and Phosphatases

Both represent strategies to control metabolism. The action of protein kinases is reversed by protein phosphatases.

Sequence of Events from Receptor to Protein Kinase

High-Yield

G protein

Receptors in these pathways are coupled through trimetric G proteins in the membrane. The 3 subunits in this type of G protein are α, β, and γ. In its inactive form, the α subunit binds GDP and is in complex with the β and γ subunits. When a hormone binds to its receptor, the receptor becomes activated and, in turn, engages the corresponding G protein (**step 1 below**). The GDP is replaced with GTP, enabling the α subunit to dissociate from the β and γ subunits (**step 2 below**).

The activated α subunit alters the activity of adenylate cyclase. If the α subunit is α_s, then the enzyme is activated; if the α subunit is α_i, then the enzyme is inhibited. The GTP in the activated α subunit will be dephosphorylated to GDP (**step 3 below**) and will rebind to the β and γ subunits (**step 4 below**), rendering the G protein inactive.

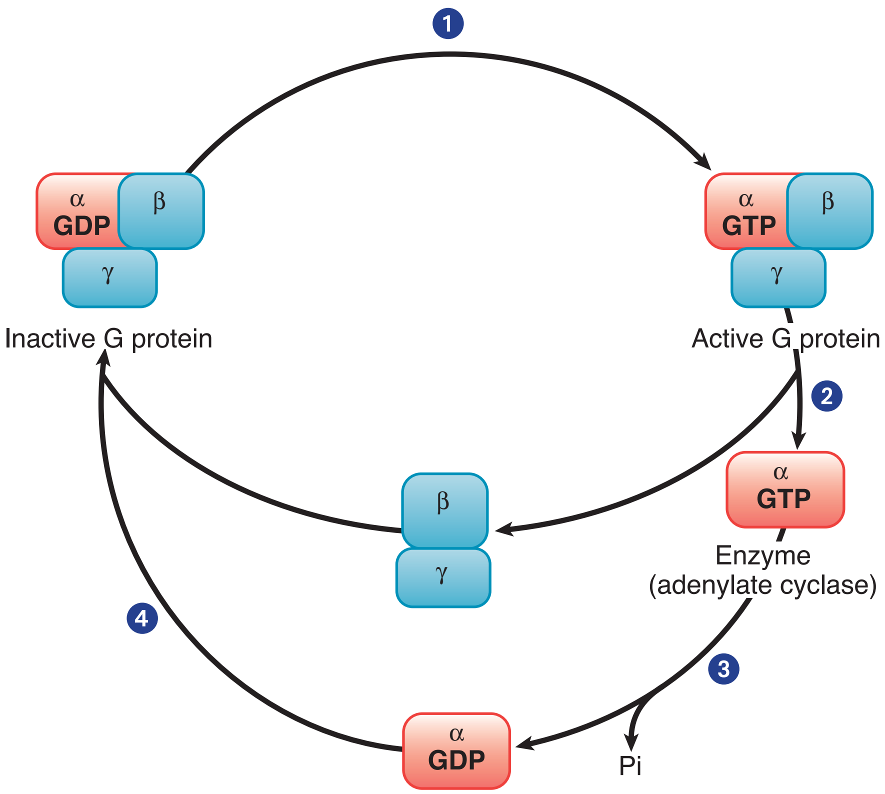


Figure I-9-2. Trimeric G Protein Cycle

Cyclic AMP (cAMP) and phosphatidylinositol bisphosphate (PIP_2)

The receptors all have characteristic 7-helix membrane-spanning domains.

The sequence of events, leading from receptor to activation of the protein kinase via the cAMP and PIP_2 second messenger systems, is as follows:

- Hormone binds receptor
- Trimeric G protein in membrane is engaged
- Enzyme (adenylate cyclase or phospholipase) activated
- Second messenger generated
- Protein kinase activated
- Protein phosphorylation (minutes) and gene expression (hours)

An example of inhibition of adenylate cyclase via G_i is epinephrine inhibition (through its binding to α_2 adrenergic receptor) of insulin release from β cells of the pancreas.

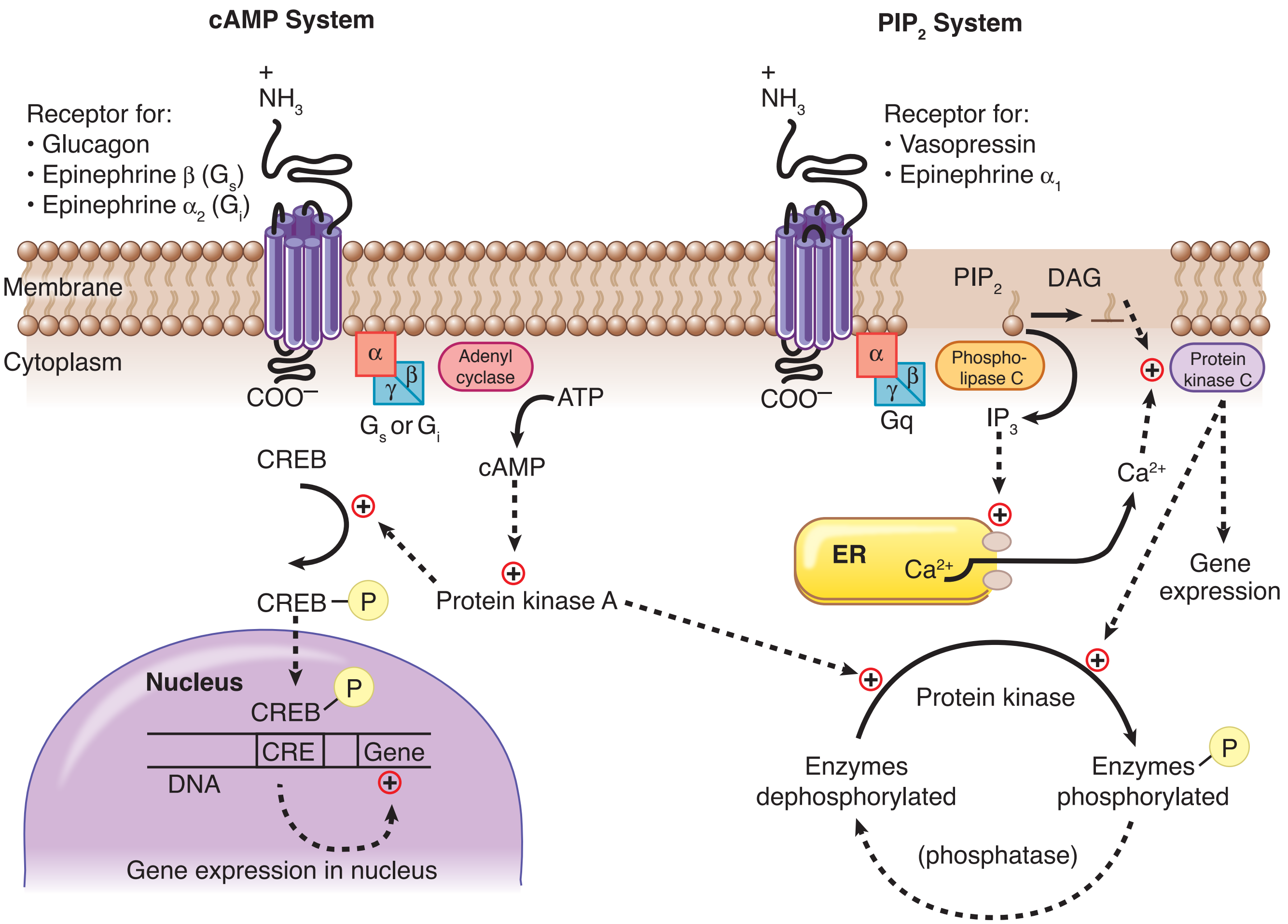


Figure I-9-3. Cyclic AMP and Phosphatidylinositol Bisphosphate (PIP_2)

cGMP

Atrial natriuretic factor (ANF), produced by cells in the atrium of the heart in response to distension, binds the ANF receptor in vascular smooth muscle and in the kidney. The ANF receptor spans the membrane and has guanylate cyclase activity associated with the cytoplasmic domain. It causes relaxation of vascular smooth muscle, resulting in vasodilation, and in the kidney it promotes sodium and water excretion.

Nitric oxide (NO) is synthesized by vascular endothelium in response to vasodilators. It diffuses into the surrounding vascular smooth muscle, where it directly binds the heme group of soluble guanylate cyclase, activating the enzyme.

Both the ANF receptor and the soluble guanylate cyclase are associated with the same vascular smooth muscle cells.

Note

Once generated, the second messengers cAMP and cGMP are slowly degraded by a class of enzymes called phosphodiesterases (PDEs).

Bridge to Microbiology

E. coli heat stable toxin (STa)

A similar guanylate cyclase receptor in enterocytes is the target of *E. coli* heat-stable toxin (STa). The toxin binds to, and stimulates, the guanylate cyclase increasing cGMP. This causes increased activity of CFTR and diarrhea.

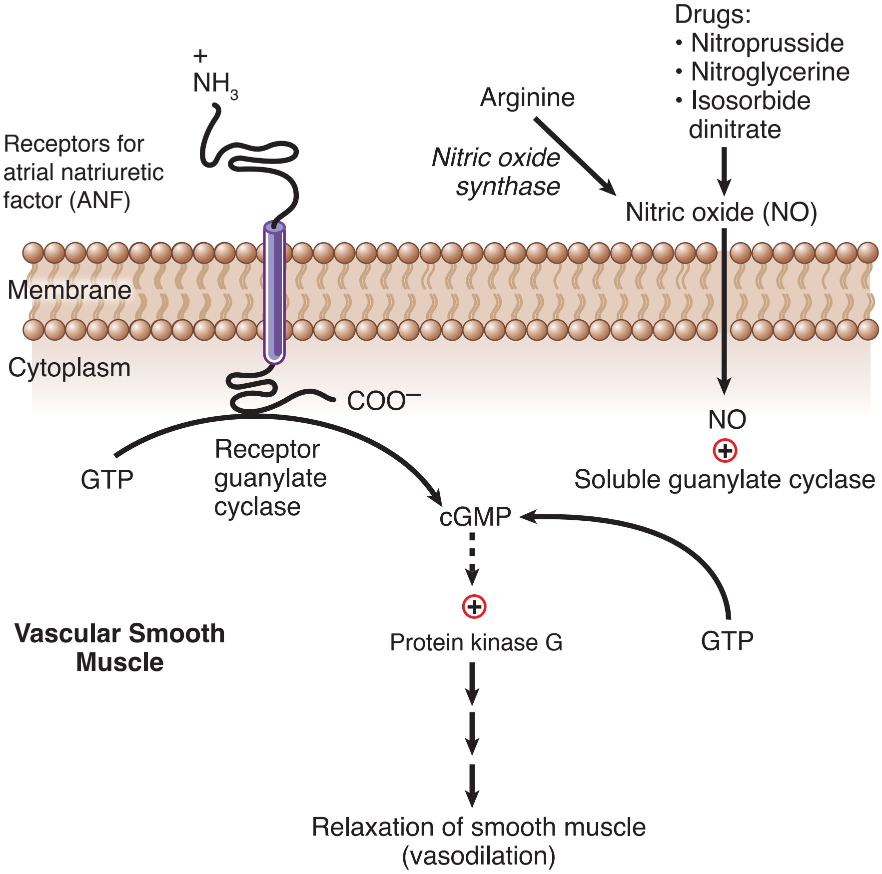


Figure I-9-4. Cyclic GMP

The sequence from receptor to protein kinase is quite similar to the one above for cAMP, with 2 important variations:

- The ANF receptor has intrinsic guanylate cyclase activity. Because no G protein is required in the membrane, the receptor lacks the 7-helix membrane-spanning domain.
- Nitric oxide diffuses into the cell and directly activates a soluble, cytoplasmic guanylate cyclase, so no receptor or G protein is required.

The Insulin Receptor: A Tyrosine Kinase

High-Yield

Insulin binding activates the tyrosine kinase activity associated with the cytoplasmic domain of its receptor. There is no trimeric G protein, enzyme, or second messenger required to activate this protein tyrosine kinase activity:

- Hormone binds receptor
- Receptor tyrosine kinase (protein kinase) is activated
- Protein phosphorylation (autophosphorylation and activation of other proteins)

Once autophosphorylation begins, a complex of other events ensues. An insulin receptor substrate (IRS-1) binds the receptor and is phosphorylated on tyrosine

residues, allowing proteins with SH2 (*src* homology) domains to bind to the phosphotyrosine residues on IRS-1 and become active. In this way, the receptor activates several enzyme cascades, which involve:

- Activation of phosphatidylinositol-3 kinase (PI-3 kinase), one of whose effects in adipose and muscle tissues is to increase GLUT-4 in the membrane
- Activation of protein phosphatases. Paradoxically, insulin stimulation via its tyrosine kinase receptor ultimately may lead to dephosphorylating enzymes
- Stimulation of the monomeric G protein ($p21^{ras}$) encoded by the normal *ras* gene

All these mechanisms can be involved in controlling gene expression, although the pathways by which this occurs have not yet been completely characterized.

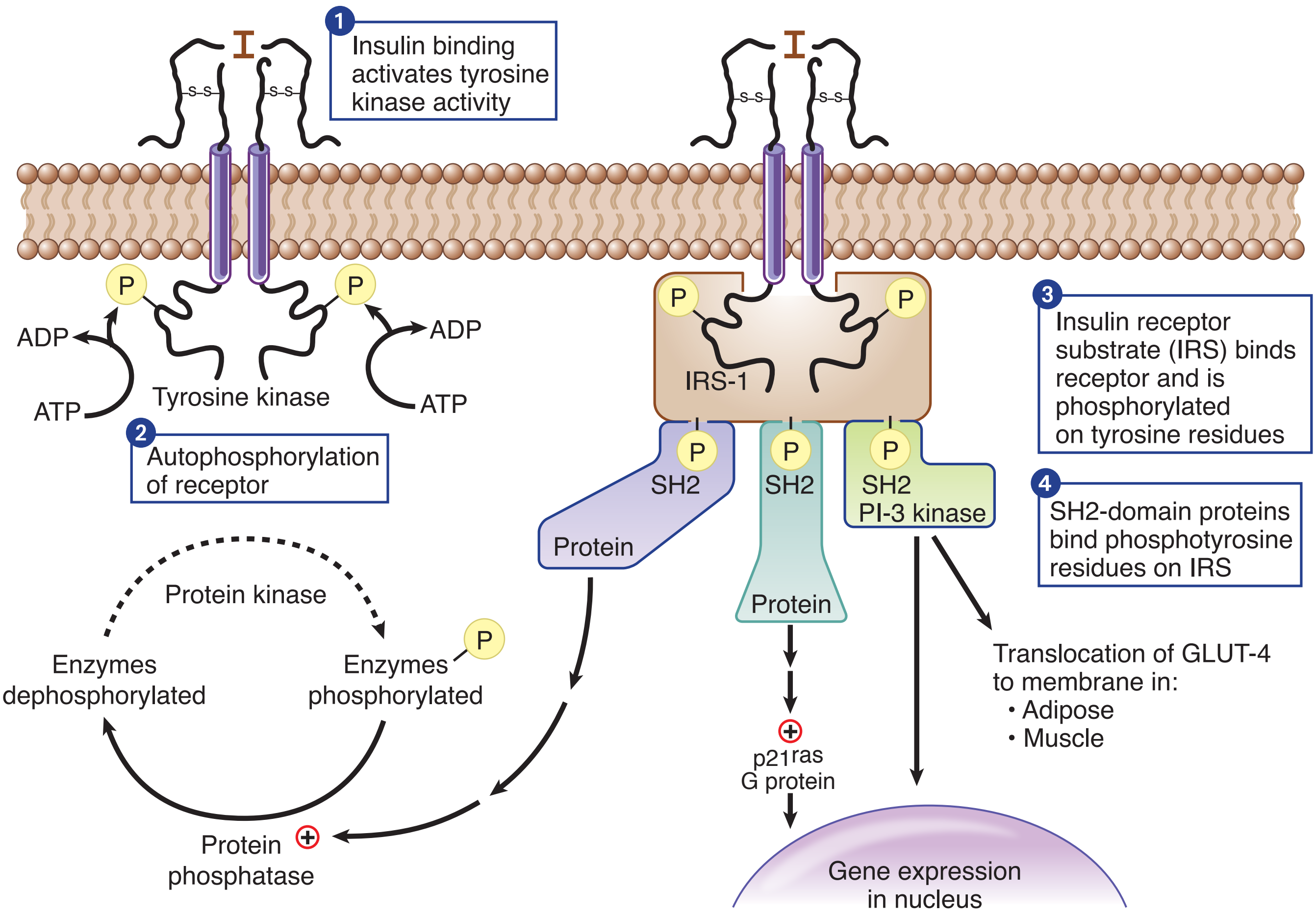


Figure I-9-5. Insulin Receptor

Tyrosine kinase receptors are also involved in signaling by several growth factors, including platelet-derived growth factor (PDGF) and epidermal growth factor (EGF).

Recall Question

Epinephrine, through inhibition of adenylate cyclase, prevents the secretion of which of the following products?

A. Gastrin

B. Glucagon

C. Insulin

D. Lipoprotein lipase

Answer: C

Functional Relationship of Glucagon and Insulin

High-Yield

Insulin (associated with well-fed, absorptive metabolism) and **glucagon** (associated with fasting and postabsorptive metabolism), usually oppose each other with respect to pathways of energy metabolism. Glucagon works through the cAMP system to activate protein kinase A favoring phosphorylation of rate-limiting enzymes, whereas insulin often activates protein phosphatases that dephosphorylate many of the same enzymes.

Glucagon promotes phosphorylation of both rate-limiting enzymes (glycogen phosphorylase for glycogenolysis and glycogen synthase for glycogen synthesis). The result is twofold in that synthesis slows and degradation increases, but both effects contribute to the same physiologic outcome, release of glucose from the liver during hypoglycemia.

Insulin reverses this pattern, promoting glucose storage after a meal. The reciprocal relationship between glucagon and insulin is manifested in other metabolic pathways, such as triglyceride synthesis and degradation.

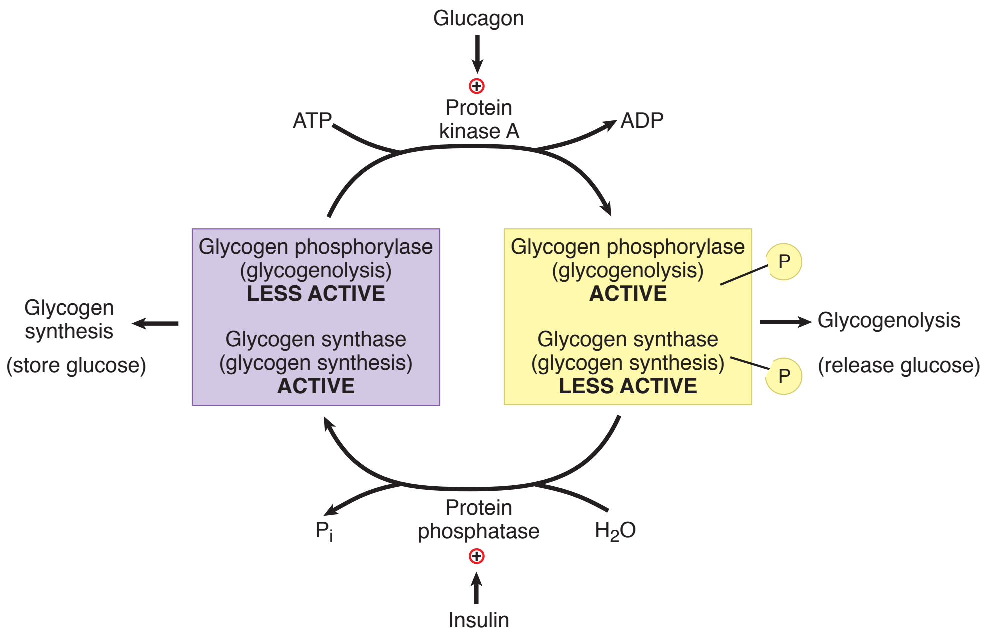


Figure I-9-6. Opposing Activities of Insulin and Glucagon

G PROTEINS IN SIGNAL TRANSDUCTION

Table I-9-2 seen earlier summarizes the major components of water-soluble hormone pathways reviewed in this section. There are several G proteins (GTP-binding) involved. Trimeric G proteins include G_s, G_i, G_q, and in the photoreceptor pathway reviewed in Chapter 10, G_t (transducin). Receptors that engage these all have the 7-helix membrane-spanning structure. Receptor stimulation causes the Gα subunit to bind GTP and become active. The Gα subunit subsequently hydrolyzes the GTP to GDP, terminating the signal. The $p21^{ras}$ G protein is monomeric.

G-protein defects can cause disease in several ways.

Table I-9-3. Abnormal G Proteins and Disease

Defect	Example	Disease
ADP-ribosylation by:		
• *Cholera* toxin	$G_s\alpha$	Diarrhea of cholera
• *E. coli* toxin	$G_s\alpha$	Traveler's diarrhea
• *Pertussis* toxin	$G_i\alpha$	Pertussis (whooping cough)
Oncogenic mutations	$p21^{ras}$ (*ras*)	Colon, lung, breast, bladder tumors

ADP-Ribosylation by Bacterial Toxins

High-Yield

Certain bacterial exotoxins are enzymes which attach the adenosine diphosphate (ADP)-ribose residue of NAD to Gα subunits, an activity known as ADP-ribosylation. In humans, some ADP-ribosylation is physiological but it may also be pathological:

- *Vibrio cholerae* exotoxin ADP-ribosylates $G_s\alpha$, leading to an increase in cAMP and subsequently chloride secretion from intestinal mucosal cells, and causing the diarrhea of cholera.
- Certain strains of *Escherichia coli* release toxins (heat labile or LT) similar to cholera toxin, producing traveler's diarrhea.
- *Bordetella pertussis* exotoxin ADP-ribosylates $G_i\alpha$, dramatically reducing its responsiveness to the receptor, thus increasing cAMP. It is not known how this relates to the persistent paroxysmal coughing symptomatic of pertussis (whooping cough).

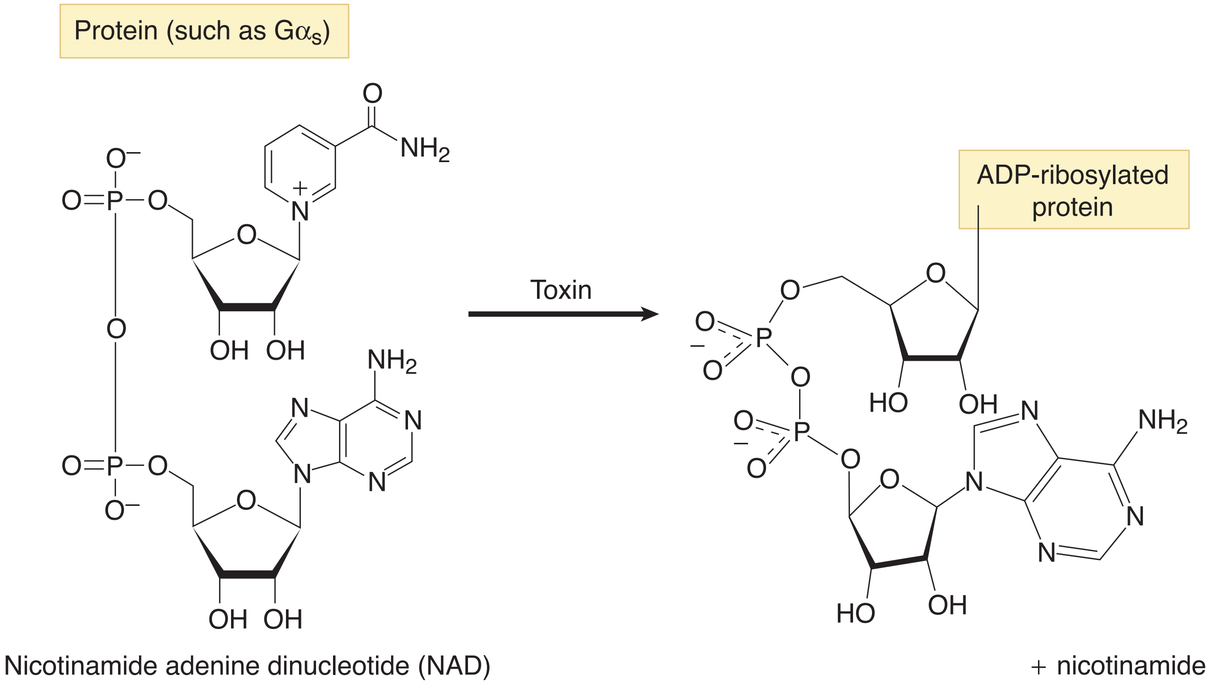


Figure I-9-7. ADP-Ribosylation of a Protein

LIPID-SOLUBLE HORMONES

Lipid-soluble hormones diffuse through the cell membrane, where they bind to their respective receptors inside the cell. The receptors have a DNA-binding domain (usually Zn-fingers) and interact with specific response elements in enhancer (or possibly silencer) regions associated with certain genes.

For example, the cortisol receptor binds to its response element in the enhancer region of the phosphoenolpyruvate carboxykinase (PEPCK) gene. By increasing the amount of PEPCK in the hepatocyte, cortisol can increase the capacity for gluconeogenesis, one of its mechanisms for responding to chronic stress often associated with injury.

The enhancer mechanism was reviewed in Chapter 5.

Review Questions

1. A patient with manic depressive disorder is treated with lithium, which slows the turnover of inositol phosphates and the phosphatidyl inositol derivatives in cells. Which of the following protein kinases is most directly affected by this drug?

   A. Protein kinase C
   B. Receptor tyrosine kinase
   C. Protein kinase G
   D. Protein kinase A
   E. Protein kinase M

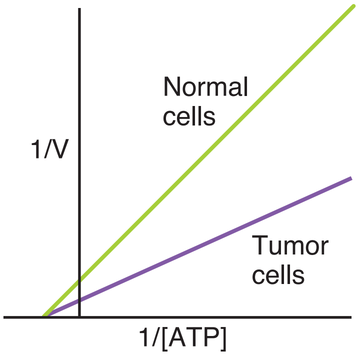
Items 2 and 3

Tumor cells from a person with leukemia have been analyzed to determine which oncogene is involved in the transformation. After partial sequencing of the gene, the predicted gene product is identified as a tyrosine kinase.

2. Which of the following proteins would most likely be encoded by an oncogene and exhibit tyrosine kinase activity?

   A. Nuclear transcriptional activator
   B. Epidermal growth factor
   C. Membrane-associated G protein
   D. Platelet-derived growth factor
   E. Growth factor receptor

3. A kinetic analysis of the tyrosine kinase activities in normal and transformed cells is shown below.

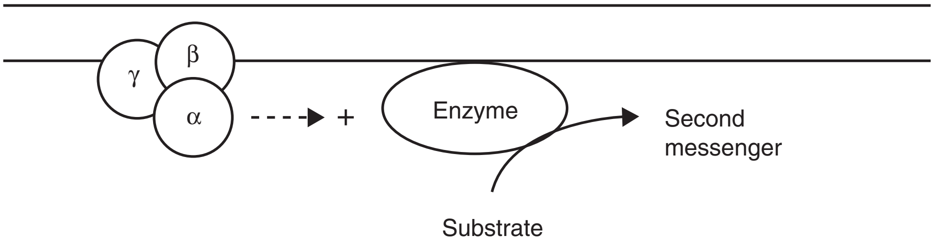


   Which of the following conclusions is best supported by these results?

   A. The tumor cell kinase has a higher-than-normal affinity for ATP
   B. A kinase gene has been deleted from the tumor cell genome
   C. A noncompetitive inhibitor has been synthesized in the tumor cells
   D. A kinase gene has been amplified in the tumor cell genome
   E. The tumor cell kinase has a lower-than-normal affinity for ATP

4. In a DNA sequencing project, an open reading frame (ORF) has been identified. The nucleotide sequence includes a coding region for an SH2 domain in the protein product. This potential protein is most likely to

   A. bind to an enhancer region in DNA
   B. be a transmembrane hormone receptor
   C. transmit signals from a tyrosine kinase receptor
   D. bind to an upstream promoter element
   E. activate a soluble guanyl cyclase enzyme in vascular smooth muscle



5. The diagram above represents a signal transduction pathway associated with hormone X. The receptor for hormone X is most likely to be characterized as a(n)

   A. seven-helix transmembrane domain receptor
   B. intracellular receptor with a zinc-finger domain
   C. helix-turn-helix transmembrane domain receptor
   D. transmembrane receptor with a guanyl cyclase domain
   E. tyrosine kinase domain receptor

6. A 58-year-old man with a history of angina for which he occasionally takes isosorbide dinitrate is having erectile dysfunction. He confides in a colleague, who suggests that sildenafil might help and gives him 3 tablets from his own prescription. The potentially lethal combination of these drugs relates to

	Isosorbide Dinitrate	Sildenafil
A.	Activates nitric oxide synthase in vascular endothelium	Inhibits guanyl cyclase in vascular smooth muscle
B.	Activates nitric oxide synthase in vascular endothelium	Inhibits guanyl cyclase in corpora cavernosa smooth muscle
C.	Releases cyanide as a byproduct	Inhibits cGMP phosphodiesterase in corpora cavernosa smooth muscle
D.	Activates guanyl cyclase in vascular smooth muscle	Inhibits cGMP phosphodiesterase in vascular smooth muscle
E.	Activates the ANF receptor in vascular smooth muscle	Inhibits protein kinase G in vascular smooth muscle

Answers

1. **Answer: A.** The description best fits the PIP_2 system in which protein kinase C is activated.

2. **Answer: E.** Although any of the listed options might be encoded by an oncogene, the "tyrosine kinase" description suggests it is likely to be a growth factor receptor.

3. **Answer: D.** Because the *y*-axis is 1/V, a smaller value for the 1/V means an increase in V_{max}. An increase in V_{max} (with no change in K_m) means an increase in the number of enzymes (a kinase in this problem). Gene amplification (insertion of additional copies of the gene in the chromosome) is a well-known mechanism by which oncogenes are overexpressed and by which resistance to certain drugs is developed. For instance, amplification of the dihydrofolate reductase gene can confer resistance to methotrexate.

4. **Answer: C.** Proteins with SH2 domains might bind to the insulin receptor substrate-1 (IRS-1) to transmit signals from the insulin receptor, a tyrosine kinase type of receptor. PI-3 kinase is an example of an SH2 domain protein. SH2 domains are not involved in DNA binding (**choices A and D**). Examples of protein domains that bind DNA include zinc fingers (steroid receptors), leucine zippers (CREB protein), and helix-turn-helix proteins (homeodomain proteins).

5. **Answer: A.** The diagram indicates that the receptor activates a trimeric G-protein associated with the inner face of the membrane and that the G-protein subsequently signals an enzyme catalyzing a reaction producing a second messenger. Receptors that activate trimeric G-proteins have a characteristic seven-helix transmembrane domain. The other categories of receptors do not transmit signals through trimeric G-proteins.

6. **Answer: D.** Nitrates may be metabolized to nitric oxide (NO) that activates a soluble guanyl cyclase in vascular smooth muscle. The increase in cGMP activates protein kinase G and subsequently leads to vasodilation. Sildenafil inhibits cGMP phosphodiesterase (PDE), potentiating vasodilation that can lead to shock and sudden death. Although sildenafil has much higher potency for the cGMP PDE isozyme in the corpora cavernosa, it can also inhibit the cGMP PDE in vascular smooth muscle. Nitric oxide synthase (**choices A and B**) is the physiologic source of nitric oxide in response to vasodilators such as acetylcholine, bradykinin, histamine, and serotonin.

Vitamins 10

Learning Objectives

- ❑ Understand differences between vitamins and coenzymes of water-soluble hormones
- ❑ Know pathologies associated with water-soluble vitamins
- ❑ Know metabolism and functions of the 4 fat-soluble vitamins

VITAMINS

Vitamins have historically been classified as water-soluble or lipid-soluble. Water-soluble vitamins are precursors for coenzymes and are reviewed in the context of the reactions for which they are important.

Table I-10-1. Water-Soluble Vitamins

Vitamin or Coenzyme	Enzyme	Pathway	Deficiency
Biotin	Pyruvate carboxylase Acetyl CoA carboxylase Propionyl CoA carboxylase	Gluconeogenesis Fatty acid synthesis Odd-carbon fatty acids, Val, Met, Ile, Thr	MCC* (rare): excessive consumption of raw eggs (contain avidin, a biotin-binding protein); also caused by biotinidase deficiency Alopecia (hair loss), bowel inflammation, muscle pain
Thiamine (B_1)	Pyruvate dehydrogenase α-Ketoglutarate dehydrogenase Transketolase Branched chain ketoacid dehydrogenase	PDH TCA cycle HMP shunt Metabolism of valine isoleucine and leucine	MCC: alcoholism (alcohol interferes with absorption) Wernicke (ataxia, nystagmus, ophthal-moplegia) Korsakoff (confabulation, psychosis) Wet beri-beri (high-output cardiac failure, fluid retention, vascular leak) and dry beri-beri (peripheral neuropathy)
Niacin (B_3) NAD(H) NADP(H)	Dehydrogenases	Many	Pellagra: diarrhea, dementia, dermatitis, and, if not treated, death Pellagra may also be related to deficiency of tryptophan (corn is low in tryptophan), which supplies a portion of the niacin requirement
Folic acid THF	Thymidylate synthase Enzymes in purine synthesis need not be memorized	Thymidine (pyrimidine) synthesis Purine synthesis	MCC: alcoholism and pregnancy (body stores depleted in 3 months), hemodialysis Homocystinemia with risk of deep vein thrombosis and atherosclerosis Megaloblastic (macrocytic) anemia Deficiency in early pregnancy causes neural tube defects in fetus
Cyanocobalamin (B12)	Homocysteine methyltransferase Methylmalonyl CoA mutase	Methionine, SAM Odd-carbon fatty acids, Val, Met, Ile, Thr	MCC: pernicious anemia. Also in aging, especially with poor nutrition, bacterial overgrowth of terminal ileum, resection of the terminal ileum secondary to Crohn disease, chronic pancreatitis, and, rarely, vegans, or infection with *D. latum* Megaloblastic (macrocytic) anemia Progressive peripheral neuropathy

*MCC, most common cause

(*Continued*)

Table I-10-1. Water-Soluble Vitamins (*continued*)

Vitamin or Coenzyme	Enzyme	Pathway	Deficiency
Pyridoxine (B_6) Pyridoxal-P (PLP)	Aminotransferases (transaminase): AST (GOT), ALT (GPT) δ-Aminolevulinate synthase	Protein catabolism Heme synthesis	MCC: isoniazid therapy Sideroblastic anemia Cheilosis or stomatitis (cracking or scaling of lip borders and corners of the mouth) Convulsions
Riboflavin (B_2) FAD(H_2)	Dehydrogenases	Many	Corneal neovascularization Cheilosis or stomatitis (cracking or scaling of lip borders and corners of the mouth) Magenta-colored tongue
Ascorbate (C)	Prolyl and lysyl hydroxylases Dopamine hydroxylase Dopamine hydroxylase	Collagen synthesis Catecholamine synthesis Absorption of iron in GI tract	MCC: diet deficient in citrus fruits and green vegetables Scurvy: poor wound healing, easy bruising (perifollicular hemorrhage), bleeding gums, increased bleeding time, painful glossitis, anemia
Pantothenic acid CoA	Fatty acid synthase Fatty acyl CoA synthetase Pyruvate dehydrogenase α-Ketoglutarate dehydrogenase	Fatty acid metabolism PDH TCA cycle	Rare

Scurvy

A 7-month-old infant presented in a "pithed frog" position, in which he lay on his back and made little attempt to lift the legs and arms because of pain. The infant cried when touched or moved, and there appeared to be numerous areas of swelling and bruising throughout the body. The mother informed the pediatrician that the infant was bottle-fed. However, the mother stated that she always boiled the formula extensively, much longer than the recommended time, to ensure that it was sterile.

The patient has infantile scurvy, which often occurs in infants 2–10 months of age who are bottle-fed with formula that is overheated for pasteurization and not supplemented with vitamin C. Vitamin C is destroyed by excessive heat. Although bleeding in an infant with scurvy might occur similarly as in an adult, gum bleeding does not unless there are erupted teeth. Biochemically, vitamin C is necessary as a cofactor by proline and lysine hydroxylases in collagen synthesis. In scurvy, because proline and lysine residues are not hydroxylated, hydrogen bonding within the triple helices does not take place. Consequently, collagen fibers are significantly less stable than normal. Vitamin C also has roles as *1*) an antioxidant, *2*) in reducing iron in the intestine to enable the absorption of iron, and *3*) in hepatic synthesis of bile acids.

Bridge to Pharmacology

High-dose niacin can be used to treat hyperlipidemia.

Note

What to Know for the Exam

Vitamins:

- Clinical manifestations of deficiencies
- Enzymes that accumulate
- Pathways
- Preventions of deficiencies
- Treatments of deficiencies

Note

Vitamin D deficiency is caused by insufficient sunlight, inadequate fortified foods (milk), or end-stage renal disease (renal osteodystrophy).

Symptoms:

- Bone demineralization
- Rickets (children)
- Osteomalacia (adults)

Vitamin A deficiency is caused by fat malabsorption or a fat-free diet.

Symptoms:

- Night blindness
- Keratinized squamous epithelia
- Xerophthalmia, Bitot spots
- Keratomalacia, blindness
- Follicular hyperkeratosis
- Alopecia

Vitamin E deficiency is caused by fat malabsorption or premature birth.

Symptoms:

- Hemolytic anemia
- Acanthocytosis
- Peripheral neuropathy
- Ataxia
- Retinitis pigmentosum

There are 4 important lipid-soluble vitamins: **D**, **A**, **K**, and **E**.

- Two of them (A and D) work through enhancer mechanisms similar to those for lipid-soluble hormones.
- In addition, all 4 have more specialized mechanisms through which they act.

Table I-10-2. Lipid-Soluble Vitamins

Vitamin	Important Functions
D (cholecalciferol)	In response to hypocalcemia, helps normalize serum calcium levels
A (carotene)	Retinoic acid and retinol act as growth regulators, especially in epithelium Retinal is important in rod and cone cells for vision
K (menaquinone, bacteria; phytoquinone, plants)	Carboxylation of glutamic acid residues in many Ca^{2+}-binding proteins, importantly coagulation factors II, VII, IX, and X, as well as protein C and protein S
E (α-tocopherol)	Antioxidant in the lipid phase. Protects membrane lipids from peroxidation

VITAMIN D AND CALCIUM HOMEOSTASIS

Hypocalcemia (below-normal blood calcium) stimulates release of parathyroid hormone (PTH), which in turn binds to receptors on cells of the renal proximal tubules. The receptors are coupled through cAMP to activation of a 1α-hydroxylase important for the final, rate-limiting step in the conversion of vitamin D to 1,25-DHCC (dihydroxycholecalciferol or calcitriol).

Once formed, 1,25-DHCC acts on duodenal epithelial cells as a lipid-soluble hormone. Its intracellular receptor (a Zn-finger protein) binds to response elements in enhancer regions of DNA to induce the synthesis of calcium-binding proteins thought to play a role in stimulating calcium uptake from the GI tract.

1,25-DHCC also facilitates calcium reabsorption in the kidney and mobilizes calcium from bone when PTH is also present. All these actions help bring blood calcium levels back within the normal range.

The relation of vitamin D to calcium homeostasis and its *in vivo* activation are shown below.

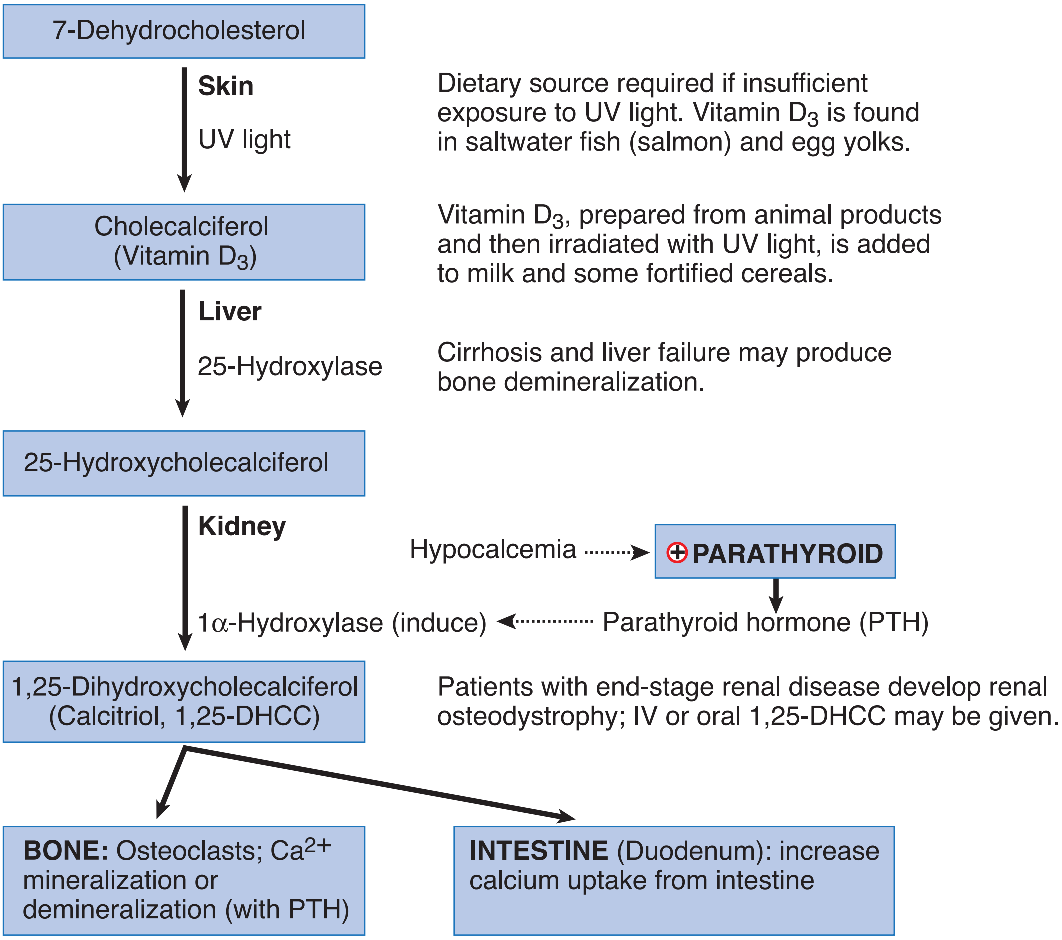


Figure I-10-1. Synthesis and Activation of Vitamin D

Synthesis of 1, 25-Dihydroxycholecalciferol (Calcitriol)

High-Yield

Humans can synthesize calcitriol from 7-dehydrocholesterol derived from cholesterol in the liver. Three steps are involved, each occurring in a different tissue:

Step 1. Activation of 7-dehydrocholesterol by UV light in the skin produces cholecalciferol (vitamin D_3); this step is insufficient for many people in cold, cloudy climates, and vitamin D_3 supplementation is necessary.

Step 2. 25-hydroxylation in the liver (patients with severe liver disease may need to be given 25-DHCC or 1,25-DHCC).

Step 3. 1α-hydroxylation in the proximal renal tubule cells in response to PTH; genetic deficiencies or patients with end-stage renal disease develop renal osteodystrophy because of insufficiency of 1,25-DHCC and must be given 1,25-DHCC or a drug analog that does not require metabolism in the kidney. Such patients include those with:

- End-stage renal disease secondary to diabetes mellitus
- Fanconi renal syndrome (renal proximal tubule defect)
- Genetic deficiency of the 1α-hydroxylase (vitamin D-resistant rickets)

Bridge to Pharmacology

Bisphosphonates are a class of drugs used in the treatment of osteoporosis.

- Function by inhibiting osteoclast action and resorption of bone; results in a modest increase in bone mineral density (BMD)
- Will lead to strengthening of bone and decrease in fractures
- Commonly used bisphosphates are ibandronate, risedronate, and alendronate

Vitamin D Toxicity

A 45-year-old man had a 3-week history of weakness, excessive urination, intense thirst, and staggering walk. For most of his adult life, he took excessive amounts of vitamin C because he was told it would help prevent the common cold. The past month, he took excessive amounts of vitamin D and calcium every day because he learned that he was developing osteoporosis. Recent lab tests revealed greatly elevated serum calcium, and vitamin D toxicity was diagnosed.

Vitamin D is highly toxic at consumption levels that continuously exceed 10× RDA, resulting in hypercalcemia. Unlike water-soluble vitamins, which are excreted in excess amounts, vitamin D can be stored in the liver as 25-hydroxycholecalciferol. The excess vitamin D can promote intestinal absorption of calcium and phosphate.

The direct effect of excessive vitamin D on bone is resorption similar to that seen in vitamin D deficiency. Therefore, the increased intestinal absorption of calcium in vitamin D toxicity contributes to hypercalcemia. Rather than help the man's osteoporosis, a large amount of vitamin D can contribute to it. Hypercalcemia can impair renal function, and early signs include polyuria, polydipsia, and nocturia. Prolonged hypercalcemia can result in calcium deposition in soft tissues, notably the kidney, producing irreversible kidney damage.

Clinical Correlate

Isotretinoin, a form of retinoic acid, is used in the treatment of acne. It is teratogenic (malformations of the craniofacial, cardiac, thymic, and CNS structures) and is therefore **absolutely contraindicated in pregnant women**. Use with caution in women of childbearing age.

Note

What to Know for the Exam

Vitamins:

- Clinical manifestations of deficiencies
- Enzymes that accumulate
- Pathways
- Preventions of deficiencies
- Treatments of deficiencies

Vitamin D Deficiency

High-Yield

Deficiency of vitamin D in childhood produces rickets, a constellation of skeletal abnormalities most strikingly seen as deformities of the legs (although many other developing bones are affected). Muscle weakness is common.

Deficiency of vitamin D after epiphyseal fusion causes osteomalacia, which produces less deformity than rickets. Osteomalacia may present as bone pain and muscle weakness.

VITAMIN A

Vitamin A (carotene) is converted to several active forms in the body associated with two important functions, maintenance of healthy epithelium and vision. Biochemically, there are 3 vitamin A structures that differ on the basis of the functional group on C-1: hydroxyl (retinol), carboxyl (retinoic acid), and aldehyde (retinal).

Maintenance of Epithelium

Retinol and retinoic acid are required for the growth, differentiation, and maintenance of epithelial cells. In this capacity they bind intracellular receptors, which are in the family of Zn-finger proteins, and they regulate transcription through specific response elements.

Vision

When first formed, all the double bonds in the conjugated double bond system in retinal are in the *trans* configuration. This form, all-*trans* retinal is not active. The conversion of all-*trans* retinal to the active form *cis*-retinal takes place in the pigmented epithelial cells. *Cis*-retinal is then transferred to opsin in the rod cells forming the light receptor rhodopsin. It functions similarly in rod and cone cells. When exposed to light, *cis*-retinal is converted all-*trans* retinal. A diagram of the signal transduction pathway for light-activated rhodopsin in the rod cell is shown in Figure I-10-2, along with the relationship of this pathway to rod cell anatomy and changes in the membrane potential. Note the following points:

- Rhodopsin is a 7-pass receptor coupled to the trimeric G protein transducin (G_t).
- When light is present, the pathway activates cGMP phosphodiesterase, which lowers cGMP.
- Rhodopsin and transducin are embedded in the disk membranes in the outer rod segment.
- cGMP-gated Na^+ channels in the cell membrane of the outer rod segment respond to the decrease in cGMP by closing and hyperpolarizing the membrane.
- The rod cell is unusual for an excitable cell in that the membrane is partially depolarized (~ –30 mV) at rest (in darkness) and hyperpolarizes on stimulation.

Because the membrane is partially depolarized in the dark, its neurotransmitter glutamate is continuously released. Glutamate inhibits the optic nerve bipolar cells with which the rod cells synapse. By hyperpolarizing the rod cell membrane, light stops the release of glutamate, relieving inhibition of the optic nerve bipolar cell and thus initiating a signal into the brain.

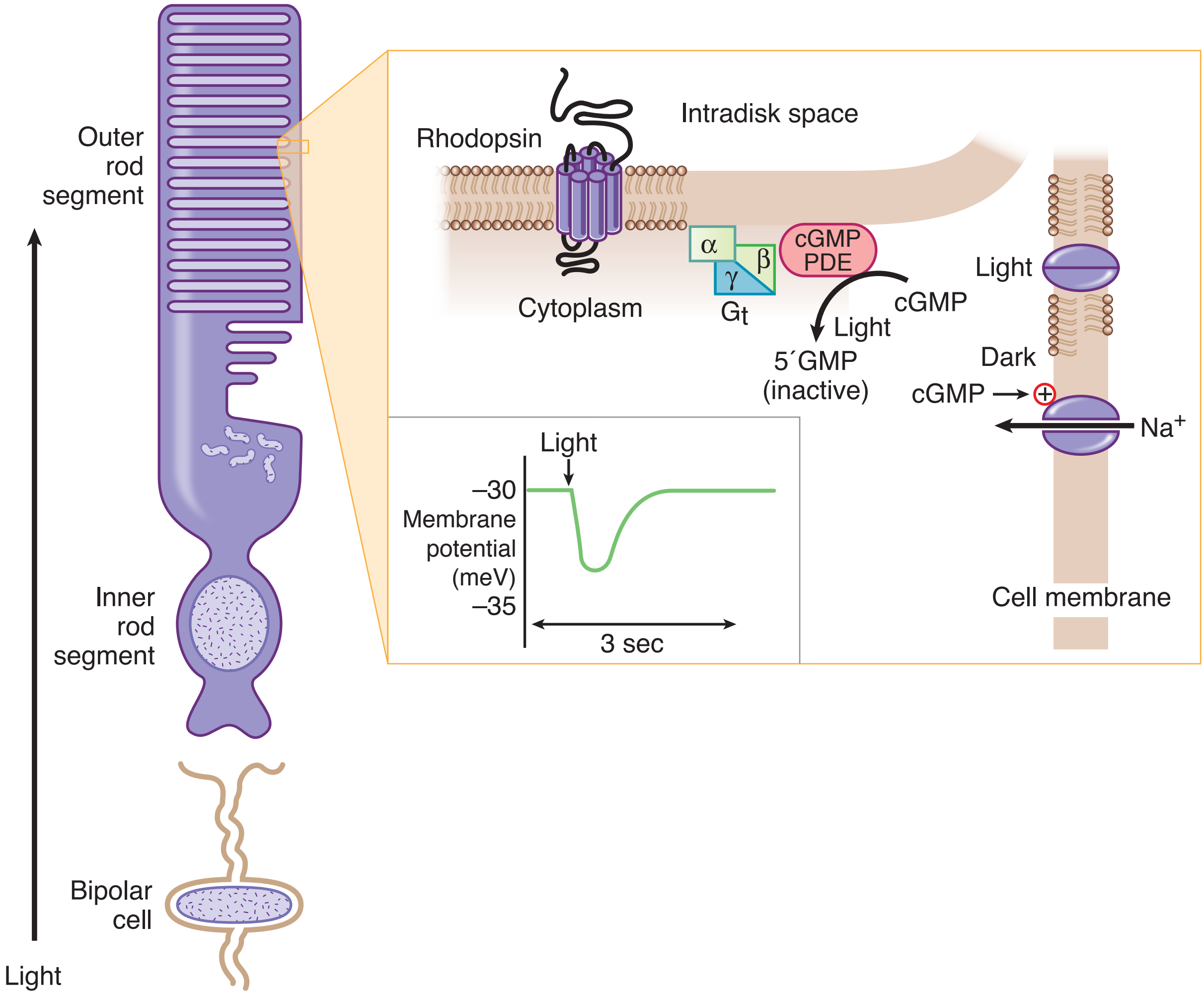


Figure I-10-2. Light-Activated Signal Transduction in the Retinal Rod Cell

Vitamin A Deficiency

A severe drought in portions of Kenya wiped out a family's yam crop, their primary food staple. Within several months, a 3-year-old child in the family began to complain of being unable to see very well, especially at dusk or at night. Also, the child's eyes were red due to constant rubbing because of dryness.

Due to the ability of the liver to store vitamin A, deficiencies which are severe enough to result in clinical manifestations are unlikely to be observed, unless there is an extreme lack of dietary vitamin A over several months. Vitamin A deficiency is the most common cause of blindness and is a serious problem in developing countries. It has a peak incidence at age 3–5. In the United States, vitamin A deficiency is most often due to fat malabsorption or liver cirrhosis.

Vitamin A deficiency results in night blindness (rod cells are responsible for vision in low light), metaplasia of the corneal epithelium, xerophthalmia (dry eyes), bronchitis, pneumonia, and follicular hyperkeratosis. The spots or patches noted in the eyes of patients with vitamin A deficiency are known as Bitot spots. Because vitamin A is important for differentiation of immune cells, deficiencies can result in frequent infections.

β-carotene is the orange pigment in yams, sweet potatoes, carrots, and yellow squash. Upon ingestion, it can be cleaved relatively slowly to two molecules of retinal by an intestinal enzyme, and each retinal molecule is then converted to all-*trans*-retinol and then absorbed by interstitial cells. Therefore, it is an excellent source of vitamin A.

Recall Question

Resection of the terminal ileum in Crohn's disease leads to deficiency of which of the following vitamins?

A. Biotin

B. Cyanocobalamin

C. Pyridoxine

D. Riboflavin

E. Thiamine

Answer: B

Note

If vitamin A is continuously ingested at levels greater than 15× RDA, toxicity develops; symptoms include excessive sweating, brittle nails, diarrhea, hypercalcemia, hepatotoxicity, vertigo, nausea, and vomiting. Unlike vitamin A, β-carotene is not toxic at high levels.

VITAMIN K

Vitamin K is required to introduce Ca^{2+} binding sites on several calcium-dependent proteins. The modification which introduces the Ca^{2+} binding site is a γ-carboxylation of glutamyl residue(s) in these proteins, often identified simply as the γ-carboxylation of glutamic acid. Nevertheless, this vitamin K-dependent carboxylation is a cotranslational modification occurring as the proteins are synthesized on ribosomes associated with the rough endoplasmic reticulum (RER) during translation.

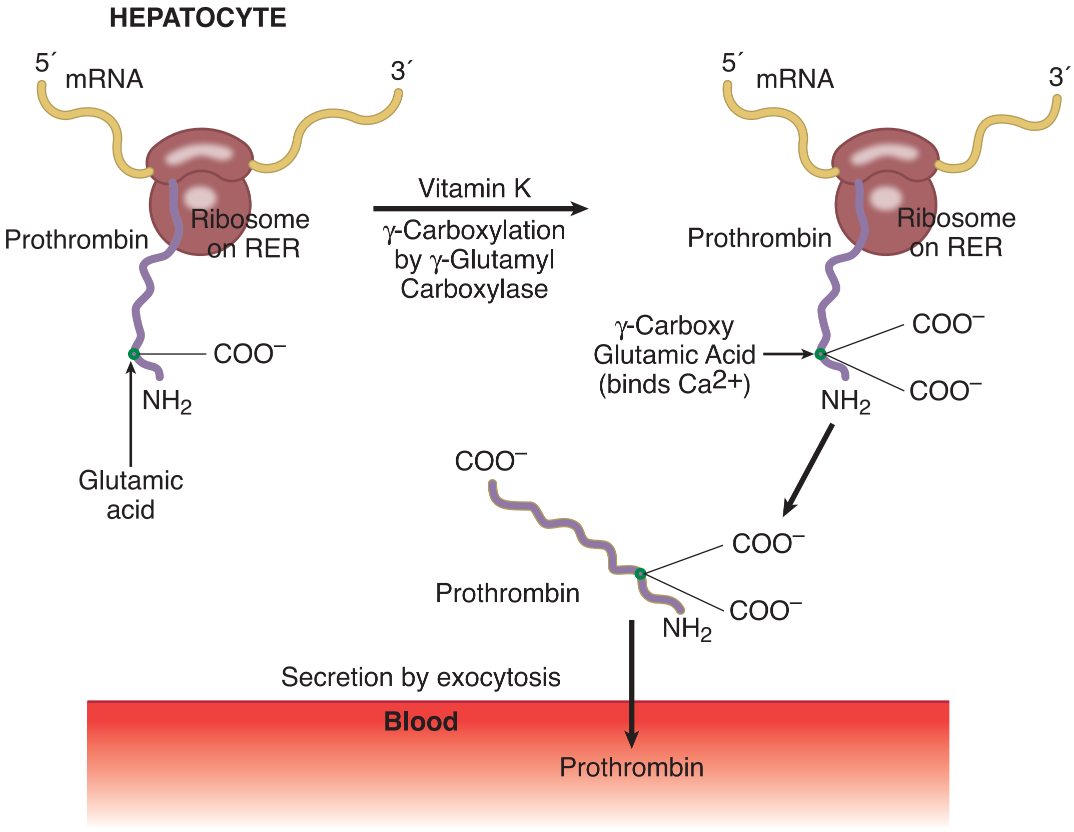


Figure I-10-3. Vitamin K–Dependent γ-Carboxylation of Prothrombin during Translation on the Rough Endoplasmic Reticulum (RER)

Examples of proteins undergoing this vitamin K–dependent carboxylation include the coagulation factors II (prothrombin), VII, IX, and X, as well as the anticoagulant proteins C and S. All these proteins require Ca^{2+} for their function.

Vitamin K deficiency produces prolonged bleeding, easy bruising, and potentially fatal hemorrhagic disease. Conditions predisposing to a vitamin K deficiency include:

- Fat malabsorption (bile duct occlusion)
- Prolonged treatment with broad-spectrum antibiotics (eliminate intestinal bacteria that supply vitamin K)
- Breast-fed newborns (little intestinal flora, breast milk very low in vitamin K), especially in a home-birth where a postnatal injection of vitamin K may not be given
- Infants whose mothers have been treated with certain anticonvulsants during pregnancy such as phenytoin (Dilantin)

Vitamin K Deficiency

A 79-year-old man living alone called his 72-year-old sister and then arrived at the hospital by ambulance complaining of weakness and having a rapid heartbeat. His sister said that he takes no medications and has a history of poor nutrition and poor hygiene. Physical examination confirmed malnourishment and dehydration. A stool specimen was positive for occult blood. He had a prolonged prothrombin time (PT), but his liver function tests (LFTs) were within normal range. He was given an injection of a vitamin that corrected his PT in 2 days.

Poor nutrition and malnourishment, lack of medications, occult blood in the stool specimen, prolonged PT, and normal LFTs are all consistent with vitamin K deficiency. Without vitamin K, several blood clotting factors (prothrombin, X, IX, VII) are not γ-carboxylated on glutamate residues by the γ-glutamyl carboxylase during their synthesis (cotranslational modification) in hepatocytes. The PT returned to normal 2 days after a vitamin K injection.

Vitamin K deficiency should be distinguished from vitamin C deficiency.

Table I-10-3. Vitamin K versus Vitamin C Deficiency

Vitamin K Deficiency	Vitamin C Deficiency
Easy bruising, bleeding	Easy bruising, bleeding
Normal bleeding time	Increased bleeding time
Increased PT	Normal PT
Hemorrhagic disease with no connective tissue problems	• Gum hyperplasia, inflammation, loss of teeth • Skeletal deformity in children • Poor wound healing • Anemia
Associated with: • Fat malabsorption • Long-term antibiotic therapy • Breast-fed newborns • Infant whose mother was taking anticonvulsant therapy during pregnancy	Associated with: • Diet deficient in citrus fruit, green vegetables

Clinical Correlate

Relative to the other proteins that undergo γ-carboxylation, protein C has a short half-life. Thus, initiation of warfarin therapy may cause a transient hypercoagulable state.

Clinical Correlate

Vitamin K (SC, IM, oral, or IV) is used to reverse bleeding from hypothrombinemia caused by excess warfarin.

Anticoagulant Therapy

High-Yield

Warfarin and dicumarol antagonize the γ-carboxylation activity of vitamin K and thus act as anticoagulants. They interfere with the cotranslational modification during synthesis of the precoagulation factors.

Once these proteins have been released into the bloodstream, vitamin K is no longer important for their subsequent activation and function.

Related to this are 2 important points:

- Warfarin and dicoumarol prevent coagulation only *in vivo* and cannot prevent coagulation of blood *in vitro* (drawn from a patient into a test tube).
- When warfarin and dicumarol are given to a patient, 2–3 days are required to see their full anticoagulant activity. Heparin or low-molecular-weight heparin is often given to provide short-term anticoagulant activity. Heparin is an activator of antithrombin III.

VITAMIN E

Vitamin E (α-tocopherol) is an antioxidant. As a lipid-soluble compound, it is especially important for protecting other lipids from oxidative damage. It prevents peroxidation of fatty acids in cell membranes, helping to maintain their normal fluidity.

Vitamin E deficiency can lead to hemolysis, neurologic problems, and retinitis pigmentosa.

High blood levels of vitamin E can cause hemorrhage in patients given warfarin.

Review Questions

Select the ONE best answer.

1. Retinitis pigmentosa (RP) is a genetically heterogeneous disease characterized by progressive photoreceptor degeneration and ultimately blindness. Mutations in more than 20 different genes have been identified in clinically affected patients. Recent studies have mapped an RP locus to the chromosomal location of a new candidate gene at 5q31. One might expect this gene to encode a polypeptide required for the activity of a(n)

    A. receptor tyrosine kinase
    B. cGMP phosphodiesterase
    C. phospholipase C
    D. adenyl cyclase
    E. protein kinase C

2. A 27-year-old woman with epilepsy has been taking phenytoin to control her seizures. She is now pregnant, and her physician is considering changing her medication to prevent potential bleeding episodes in the infant. What biochemical activity might be deficient in the infant if her medication is continued?

    A. Hydroxylation of proline
    B. Glucuronidation of bilirubin
    C. Reduction of glutathione
    D. γ-Carboxylation of glutamate
    E. Oxidation of lysine

3. A 75-year-old woman is seen in the emergency room with a fractured arm. Physical examination revealed multiple bruises and perifollicular hemorrhages, periodontitis, and painful gums. Her diet consists predominately of weak coffee, bouillon, rolls, and plain pasta. Lab results indicated mild microcytic anemia. Which of the following enzymes should be less active than normal in this patient?

    A. Homocysteine methyltransferase
    B. γ-Glutamyl carboxylase
    C. Dihydrofolate reductase
    D. ALA synthase
    E. Prolyl hydroxylase

Answers

1. **Answer: B.** Only phosphodiesterase participates as a signaling molecule in the visual cycle of photoreceptor cells.

2. **Answer: D.** Phenyl hydantoins decrease the activity of vitamin K, which is required for the γ-carboxylation of coagulation factors (II, VII, IX, X), as well as proteins C and S.

3. **Answer: E.** The patient has many signs of scurvy from a vitamin C deficiency. The diet, which contains no fruits or vegetables, provides little vitamin C. Prolyl hydroxylase requires vitamin C, and in the absence of hydroxylation, the collagen α-chains do not form stable, mature collagen. The anemia may be due to poor iron absorption in the absence of ascorbate.

Energy Metabolism 11

Learning Objectives

- ❏ Explain information related to metabolic sources of energy
- ❏ Interpret scenarios about metabolic energy storage and fuel metabolism
- ❏ Answer questions about patterns of fuel metabolism in tissues

METABOLIC SOURCES OF ENERGY

Energy is extracted from food via oxidation, resulting in the end products carbon dioxide and water. This process occurs in 4 stages.

In **stage 1**, metabolic fuels are hydrolyzed in the gastrointestinal (GI) tract to a diverse set of monomeric building blocks (glucose, amino acids, and fatty acids) and absorbed.

In **stage 2**, the building blocks are degraded by various pathways in tissues to a common metabolic intermediate, acetyl-CoA.

- Most of the energy contained in metabolic fuels is conserved in the chemical bonds (electrons) of acetyl-CoA.
- A smaller portion is conserved in reducing nicotinamide adenine dinucleotide (NAD) to NADH or flavin adenine dinucleotide (FAD) to FADH2.
- Reduction indicates the addition of electrons that may be free, part of a hydrogen atom (H), or a hydride ion (H^-).

In **stage 3**, the citric acid (Krebs, or tricarboxylic acid [TCA]) cycle oxidizes acetyl-CoA to CO_2. The energy released in this process is primarily conserved by reducing NAD to NADH or FAD to $FADH_2$.

The **final stage** is oxidative phosphorylation, in which the energy of NADH and $FADH_2$ is released via the electron transport chain (ETC) and used by an ATP synthase to produce ATP. This process requires O_2.

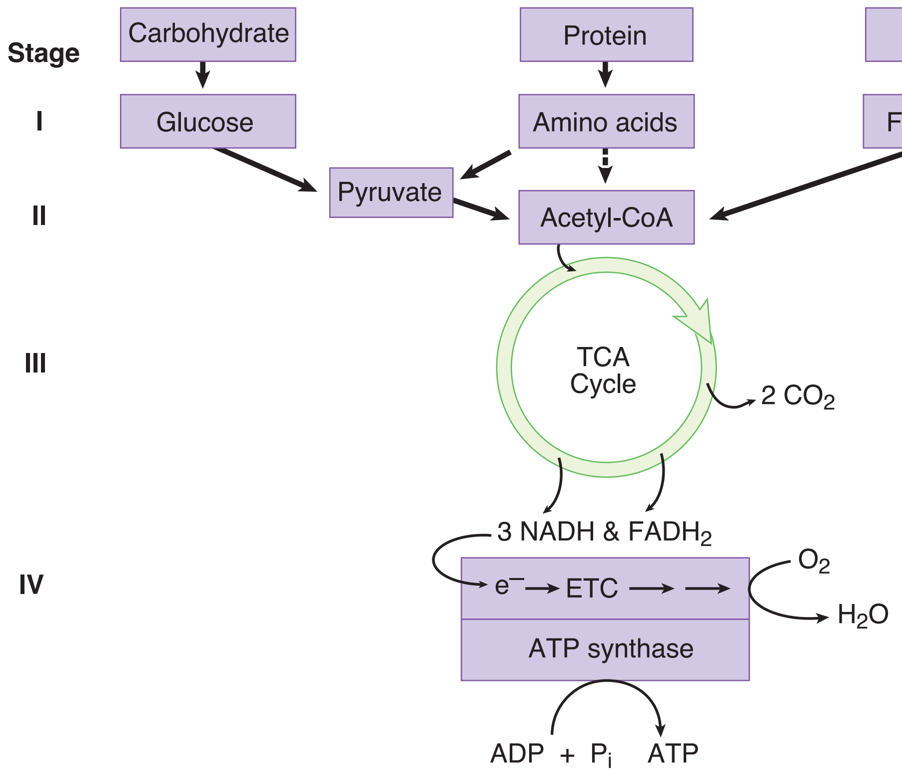


Figure I-11-1. Energy from Metabolic Fuels

METABOLIC ENERGY STORAGE

ATP is a form of circulating energy currency in cells. It is formed in catabolic pathways by phosphorylation of ADP and may provide energy for biosynthesis (anabolic pathways). There is a limited amount of ATP in circulation. Most of the excess energy from the diet is stored as fatty acids (a reduced polymer of acetyl CoA) and glycogen (a polymer of glucose). Although proteins can be mobilized for energy in a prolonged fast, they are normally more important for other functions (contractile elements in muscle, enzymes, intracellular matrix, etc.).

In addition to energy reserves, many other types of biochemicals are required to maintain an organism. Cholesterol is required for cell membrane structure, proteins for muscle contraction, and polysaccharides for the intracellular matrix, to name just a few examples. These substances may be produced from transformed dietary components.

REGULATION OF FUEL METABOLISM

The pathways that are operational in fuel metabolism depend on the nutritional status of the organism. Shifts between storage and mobilization of a particular fuel, as well as shifts among the types of fuel being used, are very pronounced in going from the well-fed state to an overnight fast, and finally to a prolonged state of starvation. The shifting metabolic patterns are regulated mainly by the insulin/glucagon ratio. Insulin is an anabolic hormone which promotes fuel

storage. Its action is opposed by a number of hormones, including glucagon, epinephrine, cortisol, and growth hormone. The major function of glucagon is to respond rapidly to decreased blood glucose levels by promoting the synthesis and release of glucose into the circulation.

Anabolic and catabolic pathways are controlled at 3 important levels:

- Allosteric inhibitors and activators of rate-limiting enzymes
- Control of gene expression by insulin and glucagon
- Phosphorylation (glucagon) and dephosphorylation (insulin) of rate-limiting enzymes

Well-Fed (Absorptive) State

High-Yield

Immediately after a meal, the blood glucose level rises and stimulates the release of insulin. The 3 major target tissues for insulin are liver, muscle, and adipose tissue. Insulin promotes glycogen synthesis in liver and muscle. After the glycogen stores are filled, the liver converts excess glucose to fatty acids and triglycerides. Insulin promotes triglyceride synthesis in adipose tissue and protein synthesis in muscle, as well as glucose entry into both tissues. After a meal, most of the energy needs of the liver are met by the oxidation of excess amino acids.

Two tissues—brain and red blood cells—are insensitive to insulin (are insulin-independent). The brain and other nerves derive energy from oxidizing glucose to CO_2 and water in both the well-fed and normal fasting states. Only in prolonged fasting does this situation change. Under all conditions, red blood cells use glucose anaerobically for all their energy needs.

Postabsorptive State

High-Yield

Glucagon and epinephrine levels rise during an overnight fast. These hormones exert their effects on skeletal muscle, adipose tissue, and liver. In liver, glycogen degradation and the release of glucose into the blood are stimulated. Hepatic gluconeogenesis is also stimulated by glucagon, but the response is slower than that of glycogenolysis. The release of amino acids from skeletal muscle and fatty acids from adipose tissue are both stimulated by the decrease in insulin and by an increase in epinephrine. The amino acids and fatty acids are taken up by the liver, where the amino acids provide the carbon skeletons and the oxidation of fatty acids provides the ATP necessary for gluconeogenesis.

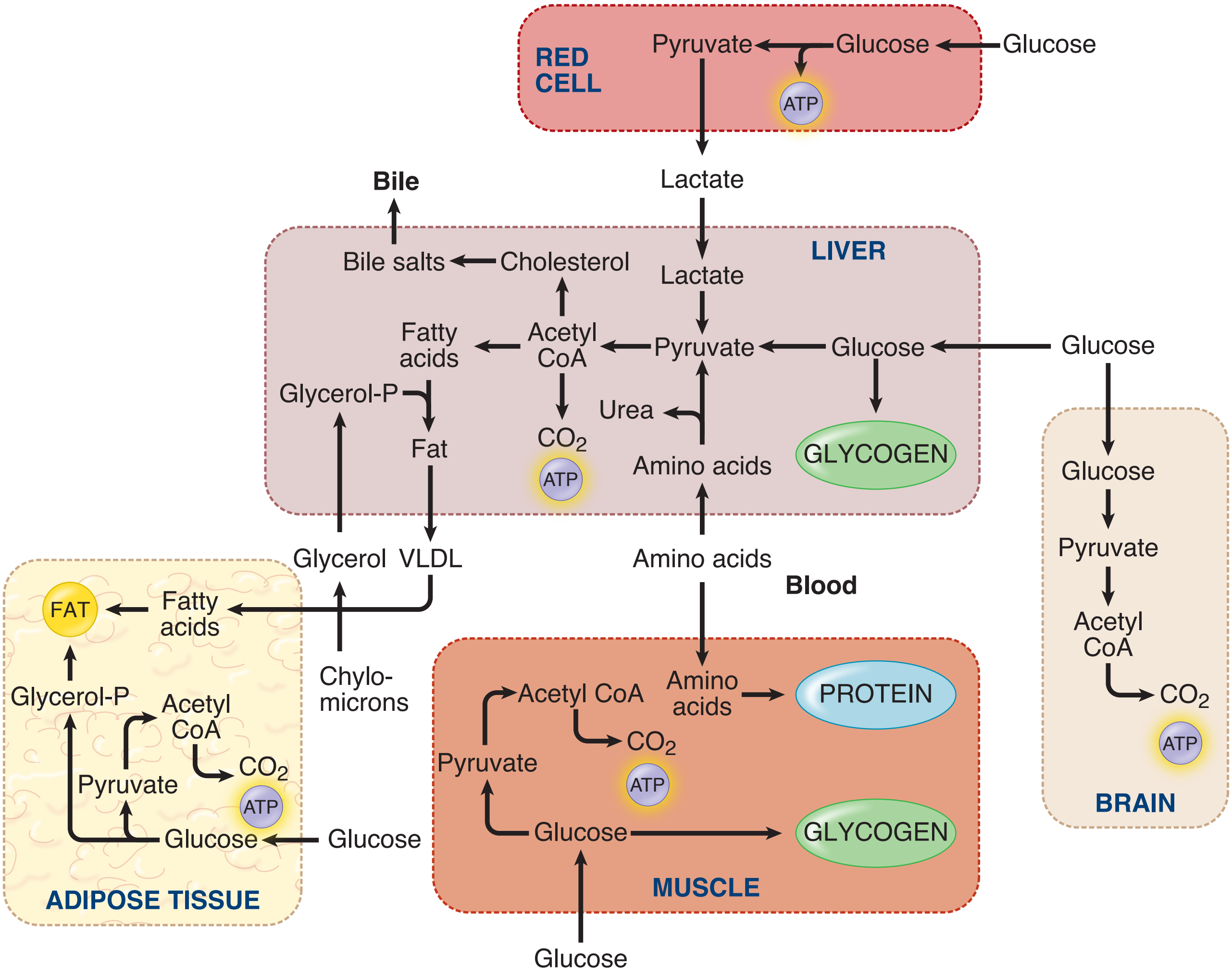


Figure I-11-2. Metabolic Profile of the Well-Fed (Absorptive) State

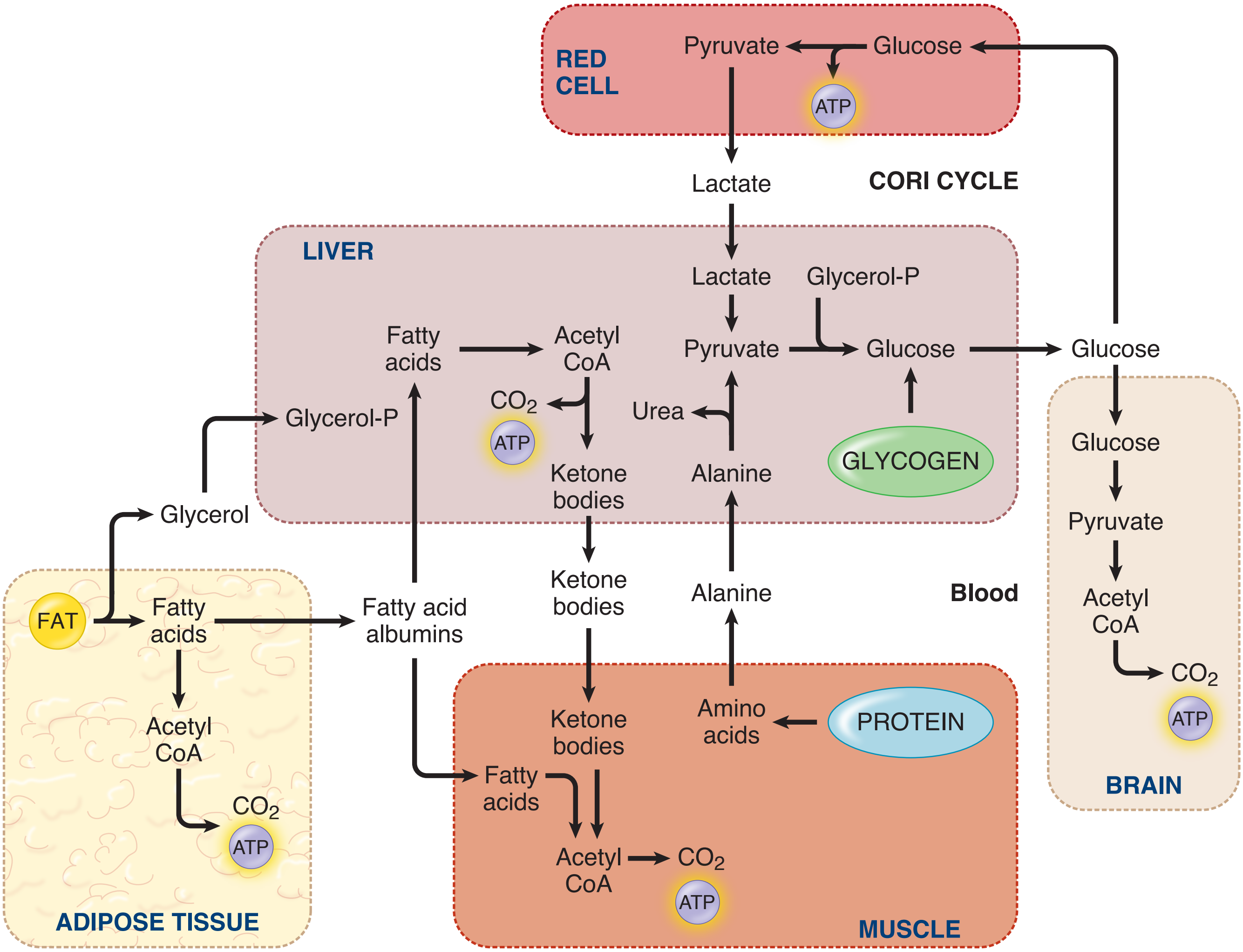


Figure I-11-3. Metabolic Profile of the Postabsorptive State

Prolonged Fast (Starvation)

High-Yield

Levels of glucagon and epinephrine are markedly elevated during starvation. Lipolysis is rapid, resulting in excess acetyl-CoA that is used for ketone synthesis. Levels of both lipids and ketones are therefore increased in the blood. Muscle uses fatty acids as the major fuel, and the brain adapts to using ketones for some of its energy.

After several weeks of fasting, the brain derives approximately 2/3 of its energy from ketones and 1/3 from glucose. The shift from glucose to ketones as the major fuel diminishes the amount of protein that must be degraded to support gluconeogenesis. There is no "energy-storage form" for protein because each protein has a specific function in the cell. Therefore, the shift from using glucose to ketones during starvation spares protein, which is essential for these other functions. Red blood cells (and renal medullary cells) that have few, if any, mitochondria continue to be dependent on glucose for their energy.

Note

Carbohydrate (4 kcal/gm)

Protein (4 kcal/gm)

Fat (9 kcal/gm)

Alcohol (7 kcal/gm)

Note

A **recommended 2,100-kcal diet** consisting of 58% carbohydrate, 12% protein, and 30% fat content:

305 g of carbohydrate

$0.58 \times 2{,}100$ kcal $= 1{,}218$ kcal

$1{,}218$ kcal/4 kcal/g $= 305$ g

63 g of protein

$0.12 \times 2{,}100 = 252$ kcal

252 kcal/4 kcal/g $= 63$ g

70 g of fat

$0.30 \times 2{,}100 = 630$ kcal

630 kcal/9 kcal/g $= 70$ g

PATTERNS OF FUEL METABOLISM IN TISSUES

Fats are much more energy-rich than carbohydrates, proteins, or ketones. Complete combustion of fat results in 9 kcal/g compared with 4 kcal/g derived from carbohydrate, protein, and ketones. The storage capacity and pathways for utilization of fuels varies by organ and nutritional status of the organism as a whole.

Table I-11-1. Preferred Fuels in the Well-Fed and Fasting States

Organ	Well-Fed	Fasting
Liver	Glucose and amino acids	Fatty acids
Resting skeletal muscle	Glucose	Fatty acids, ketones
Cardiac muscle	Fatty acids	Fatty acids, ketones
Adipose tissue	Glucose	Fatty acids
Brain	Glucose	Glucose (ketones in prolonged fast)
Red blood cells	Glucose	Glucose

Liver

Two major roles of the liver in fuel metabolism are to **maintain a constant level of blood glucose under a wide range of conditions** and to **synthesize ketones when excess fatty acids are being oxidized**.

- After a meal, the glucose concentration in the portal blood is elevated.
- The liver extracts excess glucose and uses it to replenish its glycogen stores. Any glucose remaining in the liver is then converted to acetyl CoA and used for fatty acid synthesis.

- The increase in insulin after a meal stimulates both glycogen synthesis and fatty acid synthesis in liver. The fatty acids are converted to triglycerides and released into the blood as very low-density lipoproteins (VLDLs). In the well-fed state, the liver derives most of its energy from the oxidation of excess amino acids.
- Between meals and during prolonged fasts, the liver releases glucose into the blood. The increase in glucagon during fasting promotes both glycogen degradation and gluconeogenesis.
- Lactate, glycerol, and amino acids provide carbon skeletons for glucose synthesis.

Adipose Tissue

After a meal, the elevated insulin stimulates glucose uptake by adipose tissue. Insulin also stimulates fatty acid release from VLDL and chylomicron triglyceride (triglyceride is also known as triacylglycerol).

- Lipoprotein lipase, an enzyme found in the capillary bed of adipose tissue, is induced by insulin.
- The fatty acids that are released from lipoproteins are taken up by adipose tissue and re-esterified to triglyceride for storage.
- The glycerol phosphate required for triglyceride synthesis comes from glucose metabolized in the adipocyte.
- Insulin is also very effective in suppressing the release of fatty acids from adipose tissue.
- During the fasting state, the decrease in insulin and the increase in epinephrine activate hormone-sensitive lipase in fat cells, allowing fatty acids to be released into the circulation.

Recall Question

In a prolonged state of starvation, which of the following is the major source of energy for muscles?

A. Fatty acids

B. Glucose

C. Glycogen

D. Ketones

Answer: A

Skeletal Muscle

Resting muscle

The major fuels of skeletal muscle are glucose and fatty acids. Because of the enormous bulk, skeletal muscle is the body's major consumer of fuel. After a meal, under the influence of insulin, skeletal muscle takes up glucose to replenish glycogen stores and amino acids that are used for protein synthesis. Both excess glucose and amino acids can also be oxidized for energy.

In the fasting state, resting muscle uses fatty acids derived from free fatty acids in the blood. Ketones may be used if the fasting state is prolonged. In exercise, skeletal muscle may convert some pyruvate to lactate, which is transported by blood to be converted to glucose in the liver.

Clinical Correlate

Because insulin is necessary for adipose cells to take up fatty acids from triglycerides, high triglyceride levels in the blood may be an indicator of untreated diabetes.

Active muscle

The primary fuel used to support muscle contraction depends on the magnitude and duration of exercise as well as the major fibers involved. Skeletal muscle has stores of both glycogen and some triglycerides. Blood glucose and free fatty acids also may be used.

- **Fast-twitch** muscle fibers have a high capacity for anaerobic glycolysis but are quick to fatigue. They are involved primarily in short-term, high-intensity exercise.
- **Slow-twitch** muscle fibers in arm and leg muscles are well-vascularized and primarily oxidative. They are used during prolonged, low-to-moderate intensity exercise and resist fatigue. Slow-twitch fibers and the number of their mitochondria increase dramatically in trained endurance athletes.
- Short bursts of high-intensity exercise are supported by anaerobic glycolysis drawing on stored muscle glycogen.
- During moderately high, continuous exercise, oxidation of glucose and fatty acids are both important, but after 1–3 hours of sustained continuous exercise muscle glycogen stores become depleted and the intensity of exercise declines to a rate that can be supported by oxidation of fatty acids.

Cardiac Muscle

During fetal life, cardiac muscle primarily uses glucose as an energy source, but in the postnatal period there is a major switch to β-oxidation of fatty acids. Thus, in humans, fatty acids serve as the major fuel for cardiac myocytes. When ketones are present during prolonged fasting, they are also used. Thus, not surprisingly, cardiac myocytes most closely parallel the skeletal muscle during extended periods of exercise.

In patients with cardiac hypertrophy, this situation reverses to some extent. In the failing heart, glucose oxidation increases, and β-oxidation falls.

Brain

Although the brain represents 2% of total body weight, it obtains 15% of the cardiac output, uses 20% of total O_2, and consumes 25% of the total glucose. Therefore, glucose is the primary fuel for the brain.

- Blood glucose levels are tightly regulated to maintain the concentration levels that enable sufficient glucose uptake into the brain via GLUT 1 and GLUT 3 transporters.
- Because glycogen levels in the brain are minor, normal function depends upon continuous glucose supply from the bloodstream.
- In hypoglycemic conditions (<70 mg/dL), centers in the hypothalamus sense a fall in blood glucose level, and the release of glucagon and epinephrine is triggered.
- Fatty acids cannot cross the blood–brain barrier and are therefore not used at all.
- Between meals, the brain relies on blood glucose supplied by either hepatic glycogenolysis or gluconeogenesis. Only in prolonged fasts does the brain gain the capacity to use ketones for energy, and even then ketones supply only approximately 2/3 of the fuel; the remainder is glucose.

Review Questions

Select the ONE best answer.

1. Two weeks after an episode of the flu, an 8-year-old boy with IDDM is brought to the emergency room in a coma. His breathing is rapid and deep, and his breath has a fruity odor. His blood glucose is 36.5 mM (normal: 4–6 mM [70–110 mg/dL]). The physician administers IV fluids, insulin, and potassium chloride. A rapid effect of insulin in this situation is to stimulate

   A. gluconeogenesis in the liver
   B. fatty acid release from adipose
   C. glucose transport in muscle
   D. ketone utilization in the brain
   E. glycogenolysis in the liver

2. An alcoholic has been on a 2-week drinking binge during which time she has eaten little and has become severely hypoglycemic. Which additional condition may develop in response to chronic, severe hypoglycemia?

   A. Glycogen accumulation in the liver with cirrhosis
   B. Thiamine deficiency
   C. Ketoacidosis
   D. Folate deficiency
   E. Hyperuricemia

3. After a routine physical exam and blood work, a woman with a normal weight for her height was advised that her lipid profile showed an elevation of blood triglycerides. The doctor advises the patient to lower fat consumption which disappoints her since she avidly consumes whole milk. The woman consults a nutritionist, who states that whole milk is 3.5% fat, which corresponds to approximately 11 g of fat in an 8 ounce serving. If she switches to drinking skim milk (nonfat), approximately how many additional grams of carbohydrates should she consume to make up for the loss of fat in the 8 ounce serving?

   A. 5 grams
   B. 11 grams
   C. 15 grams
   D. 25 grams
   E. 35 grams

Answers

1. **Answer: C.** Insulin increases glucose transport in only two tissues, adipose and muscle. The major site of glucose uptake is muscle, which decreases hyperglycemia. Glucose and ketone transport and metabolism are insulin independent in the brain (**choice D**). Insulin would slow gluconeogenesis (**choice A**) and fatty acid release from adipose (**choice B**). Insulin would inhibit glycogenolysis in the liver (**choice E**).

2. **Answer: C.** Severe hypoglycemia lowers the insulin level and increases glucagon. This would favor fatty acid release from the adipose and ketogenesis in the liver.

3. **Answer: D.** You are expected to know that carbohydrates have 4 Kcal/gram, proteins have 4 Kcal/gram, fat has 9 Kcal/gram, and alcohol has 7 Kcal/gram. In this question, 11 grams of fat times 9 Kcal/gram = 99 Kcal which is rounded to 100 Kcal. Dividing 100 Kcal by 4 Kcal/gram of carbohydrate is 25 grams.

Glycolysis and Pyruvate Dehydrogenase

12

Learning Objectives

- ❑ Answer questions about carbohydrate digestion
- ❑ Demonstrate understanding of glucose transport
- ❑ Understand concepts of aerobic and anaerobic glycolysis
- ❑ Explain information related to galactose metabolism
- ❑ Explain information related to fructose metabolism
- ❑ Answer questions about pyruvate dehydrogenase

OVERVIEW

All cells can carry out glycolysis. In a few tissues—most importantly red blood cells—glycolysis represents the only energy-yielding pathway available. Glucose is the major monosaccharide that enters the pathway, but others such as galactose and fructose can also be used.

The first steps in glucose metabolism in any cell are transport across the membrane and phosphorylation by kinase enzymes inside the cell to prevent it from leaving via the transporter.

CARBOHYDRATE DIGESTION

Only a very small amount of the total carbohydrates ingested are monosaccharides. Most of the carbohydrates in foods are in complex forms, such as starch (amylose and amylopectin) and the disaccharides sucrose and lactose.

- In the mouth, secreted salivary amylase randomly hydrolyzes the starch polymers to dextrins (<8–10 glucoses).
- Upon entry of food into the stomach, the acid pH destroys the salivary amylase.
- In the intestine, the dextrins are hydrolyzed to the disaccharides maltose and isomaltose.
- Disaccharides in the intestinal brush border complete the digestion process:
  - Maltase cleaves maltose to 2 glucoses
  - Isomaltase cleaves isomaltose to 2 glucoses
  - Lactase cleaves lactose to glucose and galactose
  - Sucrase cleaves sucrose to glucose and fructose

Uptake of glucose into the mucosal cells is performed by the sodium/glucose transporter, an active transport system.

GLUCOSE TRANSPORT

Glucose entry into most cells is concentration driven and independent of sodium. There are 4 major glucose transporters (GLUT), each with a different affinity for glucose coinciding with its respective physiologic role. Normal glucose concentration in peripheral blood is 4–6 mM (70–110 mg/dL).

- GLUT 1 and GLUT 3 mediate basal glucose uptake in most tissues, including brain, nerves, and red blood cells. Their high affinities for glucose ensure glucose entry even during periods of relative hypoglycemia. At normal glucose concentration, GLUT 1 and GLUT 3 are at V_{max}.
- GLUT 2, a low-affinity transporter, is in hepatocytes. After a meal, portal blood from the intestine is rich in glucose. GLUT 2 captures the excess glucose primarily for storage. When the glucose concentration drops below the K_m for the transporter, much of the remainder leaves the liver and enters the peripheral circulation. In the β-islet cells of the pancreas. GLUT-2, along with glucokinase, serves as the glucose sensor for insulin release.
- GLUT 4 is in adipose tissue and muscle, and responds to the glucose concentration in peripheral blood. The rate of glucose transport in both these tissues is increased by insulin, which stimulates the movement of additional GLUT 4 transporters to the membrane by a mechanism involving exocytosis.

Bridge to Physiology

GLUT 4 translocation to the cell membrane in skeletal muscle is stimulated by exercise. This effect, which is independent of insulin, involves a 5′ AMP-activated kinase.

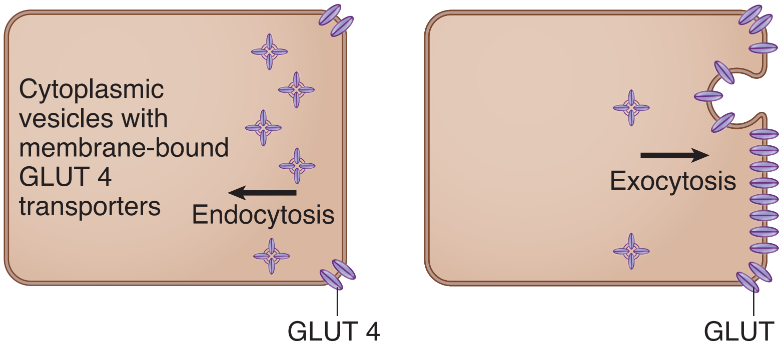


Figure I-12-1. Insulin Regulation of Glucose Transport in Muscle and Adipose Cells

Although basal transport occurs in all cells independently of insulin, the transport rate increases in adipose tissue and muscle when insulin levels rise. Muscle stores excess glucose as glycogen, and adipose tissue requires glucose to form dihydroxyacetone phosphate (DHAP), which is converted to glycerol phosphate used to store incoming fatty acids as triglyceride (TGL, 3 fatty acids attached to glycerol).

Table I-12-1. Major Glucose Transporters in Human Cells

Name	Tissues	K_m, Glucose	Functions
GLUT 1	Most tissues (brain, red cells)	~1 mM	Basal uptake of glucose
GLUT 2	Liver Pancreatic β-cells	~15 mM	Uptake and release of glucose by the liver β-cell glucose sensor
GLUT 3	Most tissues	~1 mM	Basal uptake
GLUT 4	Skeletal muscle Adipose tissue	~5 mM	Insulin-stimulated glucose uptake; stimulated by exercise in skeletal muscle

Normal blood glucose concentration is 4–6 mM (72–110 mg/dL).

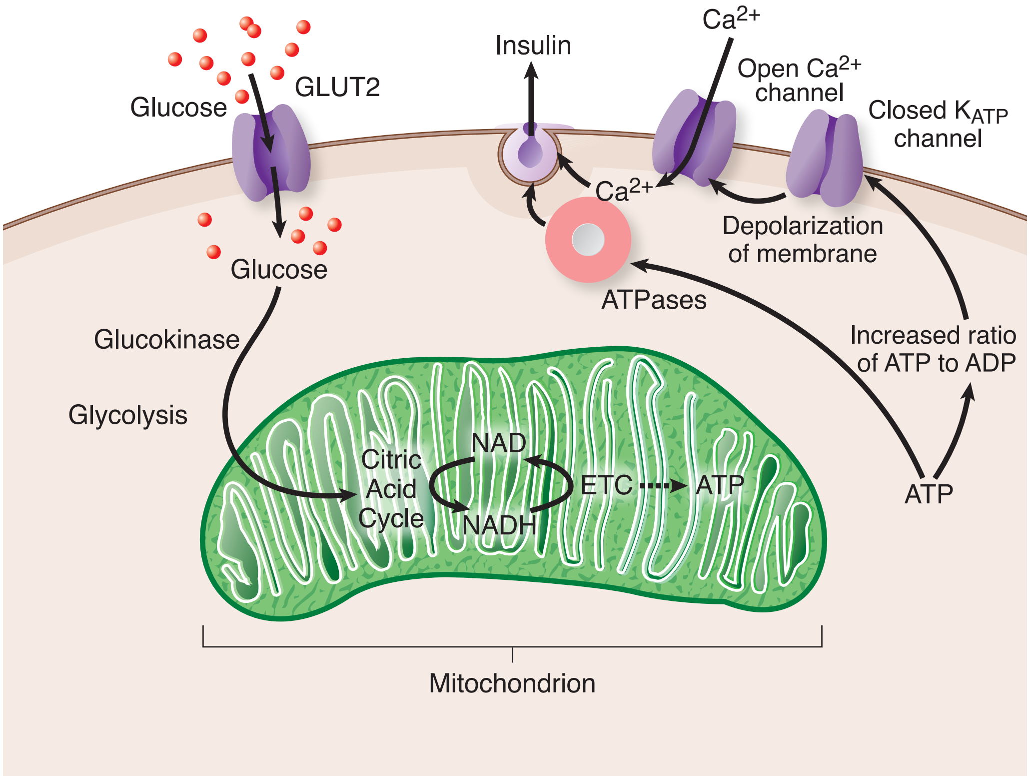


Figure I-12-2. GLUT2 and Glucokinase Together Function as the Glucose Sensor in Pancreatic β-Islet Cells

Note

Glucose induces genetic expression of the insulin gene. Insulin secretion by the pancreatic β-cells is biphasic. Glucose stimulates the first phase (within 15 minutes) with release of preformed insulin. The second phase (several hours) involves insulin synthesis at the gene level.

GLYCOLYSIS

Glycolysis is a cytoplasmic pathway that converts glucose into 2 pyruvates, releasing a modest amount of energy captured in 2 substrate-level phosphorylations and 1 oxidation reaction. If a cell has mitochondria and oxygen, glycolysis is aerobic.

If either mitochondria or oxygen is lacking, glycolysis may occur anaerobically (erythrocytes, exercising skeletal muscle), although some of the available energy is lost.

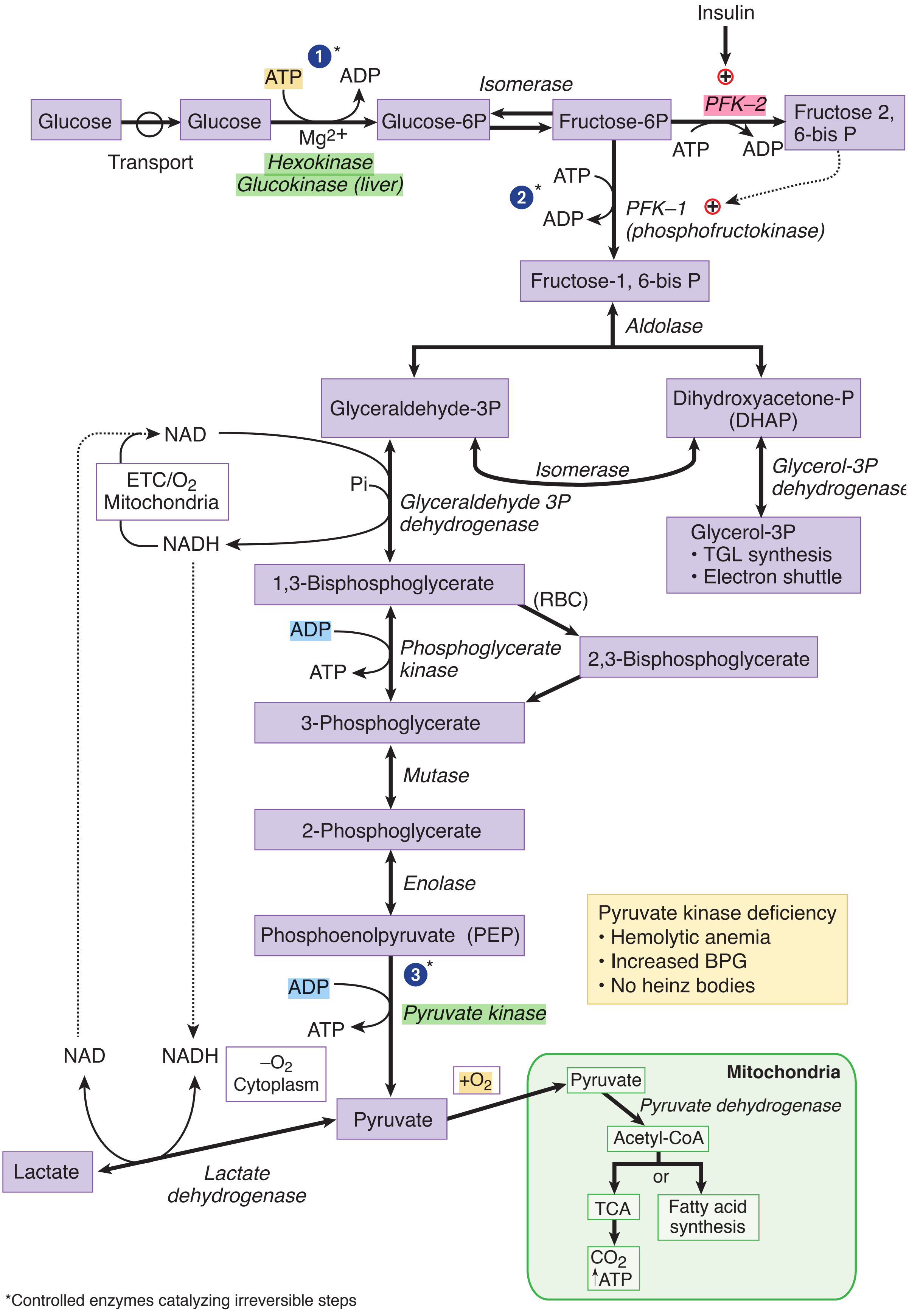


Figure I-12-3. Glycolysis

Glycolysis also provides intermediates for other pathways. In the liver, it is part of the process by which excess glucose is converted to fatty acids for storage.

Important enzymes in glycolysis include:

- **Hexokinase/glucokinase**
  - Glucose entering the cell is trapped by phosphorylation using ATP.
  - Hexokinase is widely distributed in tissues, whereas glucokinase is found only in hepatocytes and pancreatic β-islet cells.
  - Note below the differences in the Km and Vmax values. These coincide with the differences in Km values for the glucose transporters in these tissues noted earlier.

Table I-12-2. Comparison of Hexokinase and Glucokinase

Hexokinase	Glucokinase
Most tissues	Hepatocytes and pancreatic β-islet cells (along with GLUT-2, acts as the glucose sensor)
Low K_m (0.05 mM in erythrocytes)	High K_m (10 mM)
Inhibited by glucose 6-phosphate	Induced by insulin in hepatocytes

- **Phosphofructokinases (PFK-1 and PFK-2)**
  - PFK-1 is the rate-limiting enzyme and main control point in glycolysis. In this reaction, fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate using ATP.
  - PFK-1 is inhibited by ATP and citrate, and activated by AMP.
  - Insulin stimulates and glucagon inhibits PFK-1 in hepatocytes by an indirect mechanism involving PFK-2 and fructose 2,6-bisphosphate.
  - Insulin activates PFK-2 (via the tyrosine kinase receptor and activation of protein phosphatases), which converts a tiny amount of fructose 6-phosphate to fructose 2,6-bisphosphate (F2,6-BP).
  - F2,6-BP activates PFK-1.
  - Glucagon inhibits PFK-2 (via cAMP-dependent protein kinase A), lowering F2,6-BP and thereby inhibiting PFK-1.
  - PFK-1 is a multi-subunit enzyme that demonstrates cooperative kinetics.
- **Glyceraldehyde 3-phosphate dehydrogenase**
  - Glyceraldehyde 3-phosphate dehydrogenase catalyzes an oxidation and addition of inorganic phosphate (Pi) to its substrate. This results in the production of a high-energy intermediate 1,3-bisphosphoglycerate and the reduction of NAD to NADH.
  - If glycolysis is aerobic, the NADH can be reoxidized (indirectly) by the mitochondrial electron transport chain, providing energy for ATP synthesis by oxidative phosphorylation.

Note

Arsenate inhibits the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by mimicking phosphate in the reaction. The arsenate-containing product is water labile, enabling glycolysis to proceed but resulting in no ATP production.

- **3-phosphoglycerate kinase**
  - 3-phosphoglycerate kinase transfers the high-energy phosphate from 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.
  - This type of reaction, in which ADP is directly phosphorylated to ATP using a high-energy intermediate, is referred to as a **substrate-level phosphorylation**.
  - Unlike oxidative phosphorylation in mitochondria, substrate-level phosphorylations are not dependent on oxygen, and are the only means of ATP generation in an anaerobic tissue.
- **Pyruvate kinase**
  - The last enzyme in aerobic glycolysis, pyruvate kinase catalyzes a substrate-level phosphorylation of ADP using the high-energy substrate phosphoenolpyruvate (PEP).
  - Pyruvate kinase is activated by fructose 1,6-bisphosphate from the PFK-1 reaction (feed-forward activation).
- **Lactate dehydrogenase**
  - Lactate dehydrogenase is used only in anaerobic glycolysis. It reoxidizes NADH to NAD, replenishing the oxidized coenzyme for glyceraldehyde 3-phosphate dehydrogenase.
  - Without mitochondria and oxygen, glycolysis would stop when all the available NAD had been reduced to NADH. By reducing pyruvate to lactate and oxidizing NADH to NAD, lactate dehydrogenase prevents this potential problem from developing.
  - In aerobic tissues, lactate does not normally form in significant amounts. However, when oxygenation is poor (skeletal muscle during strenuous exercise, myocardial infarction), most cellular ATP is generated by anaerobic glycolysis, and lactate production increases.

Glucose Sensing in β-Islet Cells

Similar to hepatocytes of the liver, β-islet cells of the pancreas have GLUT 2 on the plasma membrane to transport glucose into the cells, as well as glucokinase to trap the incoming glucose as glucose 6-phosphate. Because both GLUT 2 and glucokinase have high K_m values for glucose, glucose is transported and phosphorylated via first-order kinetics (directly proportional to glucose concentration in the bloodstream).

> A 1-day-old infant delivered at 34 weeks' gestation due to intrauterine growth retardation developed progressive respiratory failure that required intermittent mechanical ventilation. Her blood glucose was 13.4 mM and increased to 24.6 mM. Insulin was administered to normalize her glucose. No C-peptide was detectable. Her parents were second cousins. Both had symptoms of mild diabetes controlled by diet alone. Genetic studies revealed a missense mutation (Ala378Val) in the glucokinase gene. The parents were heterozygous, and the infant homozygous, for the mutation. Recombinant mutant glucokinase showed only 0.02% of the wild-type activity.

Near-complete deficiency of glucokinase activity is associated with permanent neonatal type 1 diabetes. Glucokinase deficiency is the problem in this infant. In contrast to the case above, some mutations in the glucokinase gene alter the K_m for glucose. Those mutations which decrease the K_m (increasing the affinity for glucose) result in hyperinsulinemia and hypoglycemia. Conversely, mutations which increase the K_m (decreasing the affinity for glucose) are associated with some cases of maturity-onset diabetes of the young (MODY).

Important intermediates of glycolysis include the following:

- Dihydroxyacetone phosphate (DHAP) is used in liver and adipose tissue for triglyceride synthesis.
- 1,3-bisphosphoglycerate and phosphoenolpyruvate (PEP) are high-energy intermediates used to generate ATP by substrate-level phosphorylation.

Three enzymes in the pathway catalyze **reactions that are irreversible**. When the liver produces glucose, different reactions and thus different enzymes must be used at these 3 points:

- Glucokinase/hexokinase
- PFK-1
- Pyruvate kinase

Note

C-peptide is a short polypeptide that connects the A-chain to the B-chain in the proinsulin molecule. It is removed after proinsulin is packaged into vesicles in the Golgi apparatus.

Recall Question

Which of the following transporters increases its uptake of glucose in response to insulin?

A. Glut 1

B. Glut 2

C. Glut 3

D. Glut 4

Answer: D

ATP Production and Electron Shuttles

Anaerobic glycolysis yields 2 ATP/glucose by substrate-level phosphorylation. **Aerobic** glycolysis yields these 2 ATP/glucose plus 2 NADH/glucose that can be utilized for ATP production in the mitochondria; however, the inner membrane is impermeable to NADH.

Cytoplasmic NADH is reoxidized to NAD and delivers its electrons to one of 2 electron shuttles in the inner membrane. In the malate shuttle, electrons are passed to mitochondrial NADH and then to the electron transport chain. In the glycerol phosphate shuttle, electrons are passed to mitochondrial $FADH_2$.

- Cytoplasmic NADH oxidized using the **malate shuttle** produces a mitochondrial NADH and yields approximately 3 ATP by oxidative phosphorylation.
- Cytoplasmic NADH oxidized by the **glycerol phosphate shuttle** produces a mitochondrial $FADH_2$ and yields approximately 2 ATP by oxidative phosphorylation.

Glycolysis in the Erythrocyte

In red blood cells, anaerobic glycolysis represents the only pathway for ATP production, yielding a net 2 ATP/glucose.

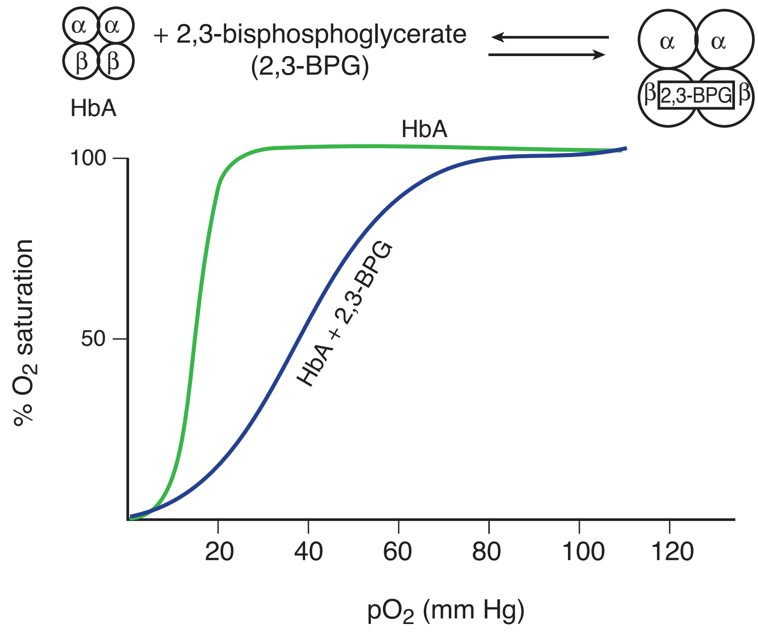


Figure I-12-4. Effect of 2,3-Bisphosphoglycerate on Hemoglobin A

Erythrocytes have bisphosphoglycerate mutase, which produces 2,3-bisphosphoglycerate (BPG) from 1,3-BPG in glycolysis.

- 2,3-BPG binds to the β-chains of hemoglobin A (HbA) and decreases its affinity for oxygen.
- This effect of 2,3-BPG is seen in the oxygen dissociation curve for HbA. The rightward shift in the curve is sufficient to allow unloading of oxygen in tissues, but still allows 100% saturation in the lungs.
- An abnormal increase in erythrocyte 2,3-BPG might shift the curve far enough so HbA is not fully saturated in the lungs.
- Although 2,3-BPG binds to HbA, it does not bind well to HbF ($\alpha_2\gamma_2$), with the result that HbF has a higher affinity for oxygen than maternal HbA, allowing transplacental passage of oxygen from mother to fetus.

Pyruvate kinase deficiency is the second most common genetic deficiency that causes a hemolytic anemia (glucose 6-phosphate dehydrogenase, G6PDH, is the most common). Characteristics include:

- Chronic hemolysis
- Increased 2,3-BPG and therefore a lower-than-normal oxygen affinity of HbA
- Absence of Heinz bodies (Heinz bodies are more characteristic of G6PDH deficiency)

The red blood cell has no mitochondria and is totally dependent on anaerobic glycolysis for ATP. In pyruvate kinase deficiency, the decrease in ATP causes the erythrocyte to lose its characteristic biconcave shape and signals its destruction in the spleen. In addition, decreased ion pumping by Na^+/K^+-ATPase results in loss of ion balance and causes osmotic fragility, leading to swelling and lysis.

Bridge to Physiology

Adaptation to high altitudes (low PO_2) involves:

- Increased respiration
- Respiratory alkalosis
- Lower P_{50}
- for hemoglobin (initial)
- Increased rate of glycolysis
- Increased [2,3-BPG] in RBC (12–24 hours)
- Normal P_{50} for hemoglobin restored by the increased level of 2,3-BPG
- Increased hemoglobin and hematocrit (days–weeks)

Clinical Correlate

Transfused blood has lower than the expected 2,3-BPG levels, making it less efficient at delivering oxygen to peripheral tissues.

GALACTOSE METABOLISM

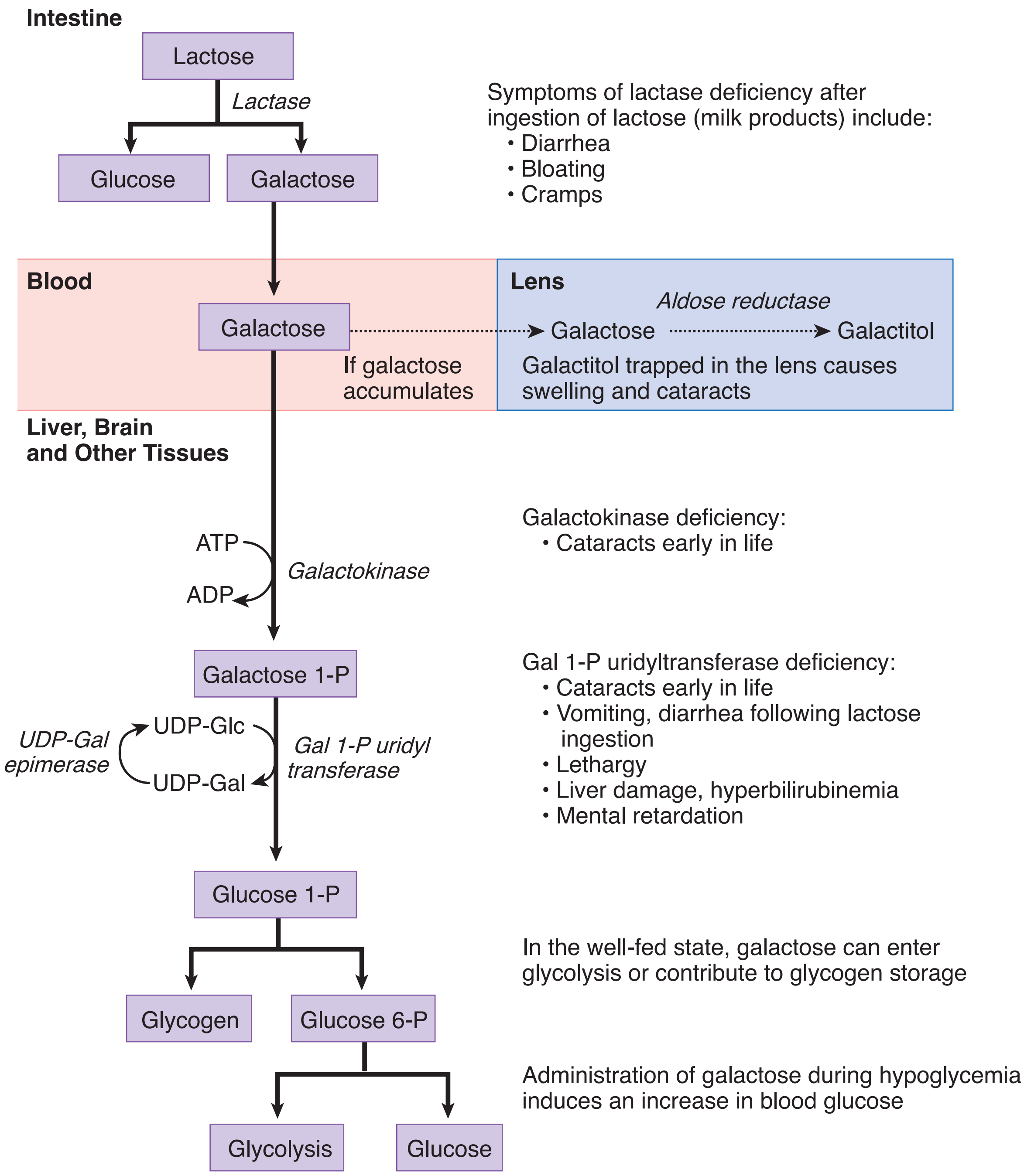


Figure I-12-5. Galactose Metabolism

An important source of galactose in the diet is the disaccharide **lactose** present in milk. Lactose is hydrolyzed to galactose and glucose by lactase associated with the brush border membrane of the small intestine. Along with other monosaccharides, galactose reaches the liver through the portal blood.

Once transported into tissues, galactose is phosphorylated (galactokinase), trapping it in the cell. Galactose 1-phosphate is converted to glucose 1-phosphate by galactose 1-P uridyltransferase and an epimerase.

Important enzymes to remember are **galactokinase** and **galactose 1-phosphate uridyltransferase**.

- Genetic deficiencies of these enzymes produce galactosemia. Cataracts, a characteristic finding in patients with galactosemia, result from conversion of the excess galactose in peripheral blood to galactitol in the lens of the eye, which has aldose reductase.
- Accumulation of galactitol in the lens causes osmotic damage and cataracts.
- The same mechanism accounts for the cataracts in diabetics because aldose reductase also converts glucose to sorbitol, which causes osmotic damage.
- Deficiency of galactose 1-phosphate uridyltransferase produces a more severe disease because, in addition to galactosemia, galactose 1-P accumulates in the liver, brain, and other tissues.

Galactosemia

Galactosemia is an autosomal recessive trait resulting from a defective gene encoding galactokinase or galactose 1-P uridyltransferase. There are over 100 heritable mutations that can cause it (incidence 1/60,000 births). Galactose will be present in elevated amounts in the blood and urine and can result in decreased glucose synthesis and hypoglycemia.

> The parents of a 2-week-old infant being breastfed returned to the hospital because the infant frequently vomited, had a persistent fever, and looked yellow since birth. The physician quickly observed that the infant had early hepatomegaly and cataracts. Blood and urine tests were identified elevated sugar (galactose and, to a smaller extent, galactitol) in the blood and urine. The doctor told the parents to bottle-feed the infant with lactose-free formula supplemented with sucrose. Subsequently, the infant improved.

Galactosemia symptoms often begin around day 3 in a newborn and include the hallmark cataracts. Jaundice and hyperbilirubinemia do not resolve if the infant is treated with phototherapy. In the galactosemic infant, the liver, which is the site of bilirubin conjugation, develops cirrhosis. Vomiting and diarrhea occur after milk ingestion because although lactose in milk is hydrolyzed to glucose and galactose by lactase in the intestine, the galactose is not properly metabolized. Severe bacterial infections (*E. coli* sepsis) are common in untreated galactosemic infants. Failure to thrive, lethargy, hypotonia, and mental retardation are other common and apparent features. Many U.S. states have mandatory screening of newborns for galactosemia. If an infant is correctly diagnosed within the first several weeks of life through a newborn screening heel prick test, formulas containing galactose-free carbohydrates are given. The life expectancy will then be normal with an appropriate diet.

Clinical Correlate

Lactose Intolerance

Primary lactose intolerance is caused by a hereditary deficiency of lactase, most commonly found in persons of Asian and African descent. Secondary lactose intolerance can be precipitated at any age by gastrointestinal disturbances such as celiac sprue, colitis, or viral-induced damage to intestinal mucosa, which is why kids with diarrhea should drink clear liquids, and not milk.

Common symptoms of lactose intolerance include vomiting, bloating, explosive and watery diarrhea, cramps, and dehydration. The symptoms can be attributed to bacterial fermentation of lactose to a mixture of CH_4, H_2, and small organic acids. The acids are osmotically active and result in the movement of water into the intestinal lumen.

Diagnosis is based on a positive hydrogen breath test after an oral lactose load. Treatment is by dietary restriction of milk and milk products (except unpasteurized yogurt, which contains active *Lactobacillus*) or by lactase pills.

FRUCTOSE METABOLISM

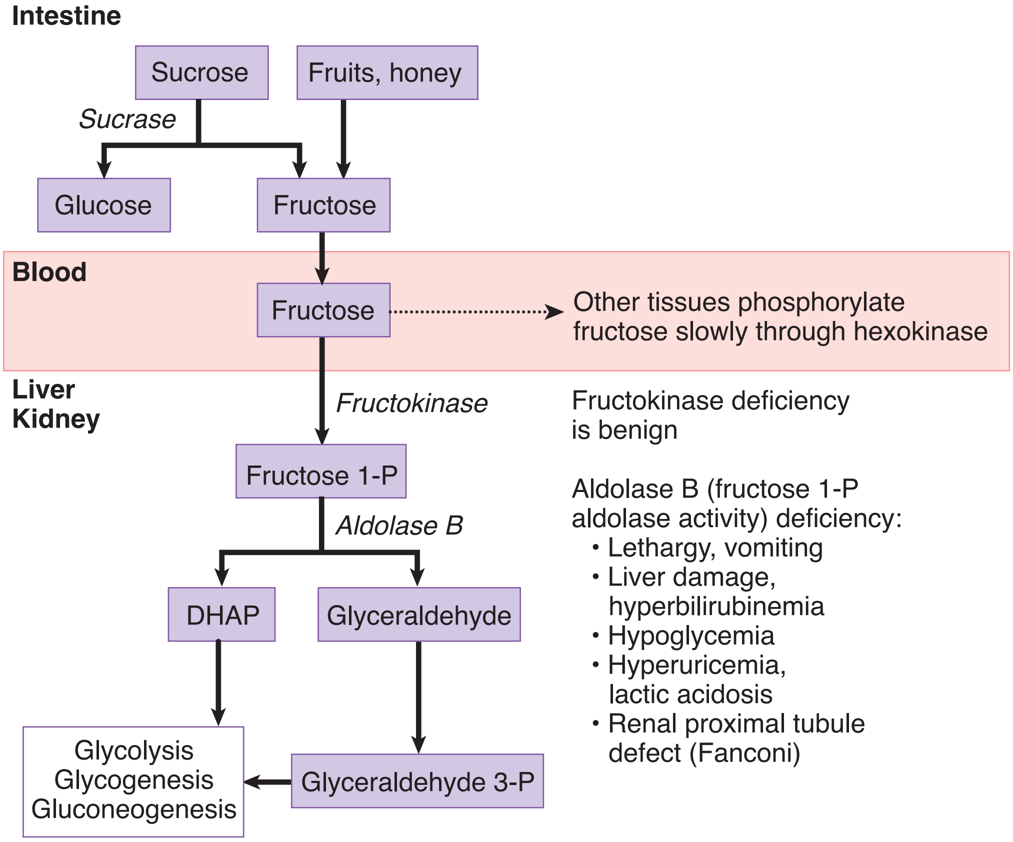


Figure I-12-6. Fructose Metabolism

Note

Because dihydroxyacetone phosphate and glyceraldehyde (the products of fructose metabolism) are downstream from the key regulatory and rate-limiting enzyme of glycolysis (PFK-1), a high-fructose drink supplies a quick source of energy in both aerobic and anaerobic cells.

Fructose is found in honey and fruit and as part of the disaccharide sucrose (common table sugar). Sucrose is hydrolyzed by intestinal brush border sucrase, and the resulting monosaccharides, glucose and fructose, are absorbed into the portal blood. The liver phosphorylates fructose and cleaves it into glyceraldehyde and DHAP. Smaller amounts are metabolized in renal proximal tubules.

Important enzymes to remember are **fructokinase** and **fructose 1-P aldolase** (aldolase B).

- Genetic deficiency of fructokinase is benign and often detected incidentally when urine is checked for glucose with a dipstick.
- Fructose 1-phosphate aldolase deficiency is severe because of accumulation of fructose 1-phosphate in the liver and renal proximal tubules. Symptoms are reversed after removing fructose and sucrose from the diet. Cataracts are not a feature of this disease because fructose is not an aldose sugar and therefore not a substrate for aldose reductase in the lens.

Hereditary Fructose Intolerance

Hereditary fructose intolerance is an autosomal recessive disease (incidence 1/20,000) due to a defect in the gene that encodes aldolase B in fructose metabolism. In the absence of the enzyme, fructose challenge results in an accumulation of fructose 1-phosphate in hepatocytes and thereby sequestering of inorganic phosphate in this substance. The drop in phosphate levels prevents its use in other pathways, such as glycogen breakdown and gluconeogenesis. Eventually, the liver becomes damaged due to the accumulation of trapped fructose 1-phosphate.

> A 4-month-old infant was breastfed and developing normally. The mother decided to begin the weaning process and started to feed the baby with fruit juices. Within a few weeks, the child became lethargic and yellow-skinned, vomited frequently, and had frequent diarrhea. The mother thought that the child might have had a food allergy and took the child to a clinic for testing. It found that the child had sugar in the urine but did not react with the glucose dipsticks.

If diagnosed early to alleviate complications, a person with fructose intolerance on a diet that excludes fructose and sucrose will develop normally and have a normal lifespan. However, complete exclusion of these sugars is difficult, especially with their widespread use as nutrients and sweeteners. Failure to correct the diet and prolonged fructose ingestion could eventually lead to proximal renal disorder resembling Fanconi syndrome.

PYRUVATE DEHYDROGENASE

Pyruvate from aerobic glycolysis enters mitochondria, where it may be converted to acetyl-CoA for entry into the citric acid cycle if ATP is needed, or for fatty acid synthesis if sufficient ATP is present. The pyruvate dehydrogenase (PDH) reaction is irreversible and cannot be used to convert acetyl-CoA to pyruvate or to glucose. PDH in the liver is activated by insulin, whereas in the brain and nerves the enzyme (actually, a complex of 5 enzymatic activities) is not responsive to hormones.

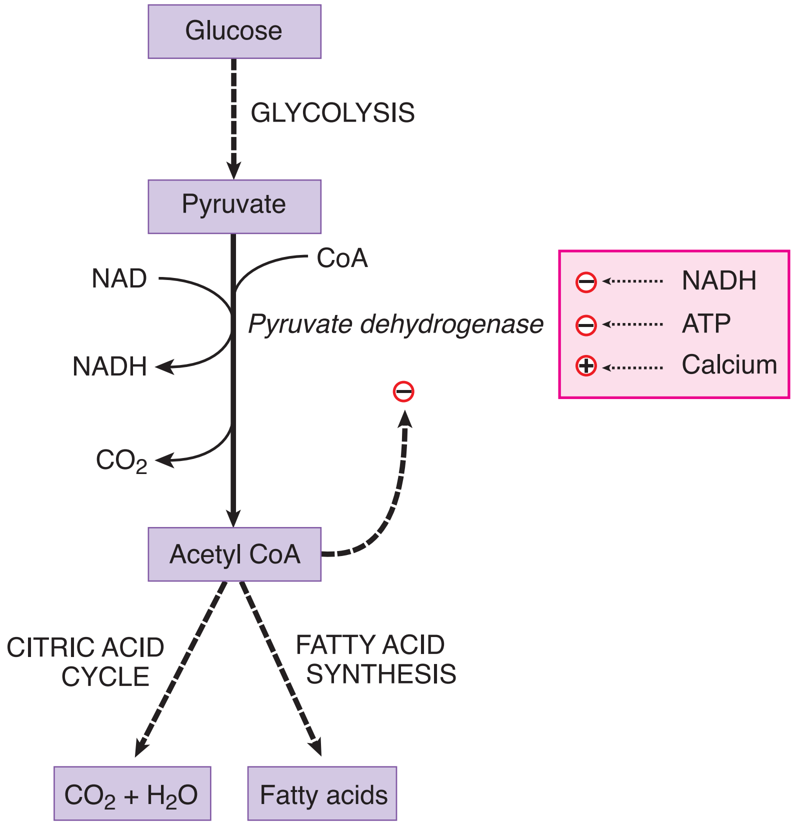


Figure I-12-7. Pyruvate Dehydrogenase

Cofactors and coenzymes used by pyruvate dehydrogenase include:

- Thiamine pyrophosphate (TPP) from the vitamin thiamine
- Lipoic acid
- Coenzyme A (CoA) from pantothenate
- FAD(H_2) from riboflavin
- NAD(H) from niacin (some may be synthesized from tryptophan)

Pyruvate dehydrogenase is inhibited by its product acetyl-CoA. This control is important in several contexts and should be considered along with pyruvate carboxylase, the other mitochondrial enzyme that uses pyruvate (introduced in gluconeogenesis, Chapter 14).

Thiamine Deficiency

High-Yield

Thiamine deficiency is commonly seen in alcoholics, since alcohol interferes with thiamine absorption from the intestine. Patients may develop a complex of symptoms associated with Wernicke peripheral neuropathy and Korsakoff psychosis. Symptoms include:

- Ataxia
- Ophthalmoplegia, nystagmus
- Memory loss and confabulation
- Cerebral hemorrhage

Congestive heart failure may be a complication (wet beri-beri) owing to inadequate ATP and accumulation of ketoacids in the cardiac muscle.

Similar to pyruvate dehydrogenase, 2 enzyme complexes use thiamine:

- α-ketoglutarate dehydrogenase (citric acid cycle)
- Branched-chain ketoacid dehydrogenase (metabolism of branched-chain amino acids)

Thiamine deficiency significantly impairs glucose oxidation, causing highly aerobic tissues (e.g., brain and cardiac muscle) to fail first. In addition, branched-chain amino acids are sources of energy in brain and muscle.

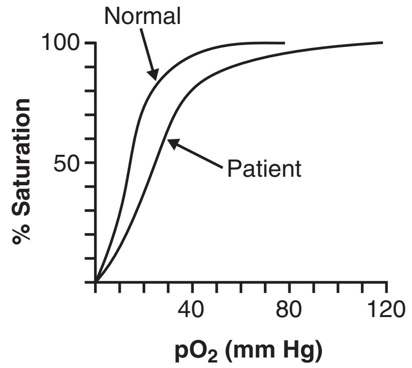
Clinical Correlate

If thiamine deficiency is suspected, give IV thiamine prior to glucose (dextrose) administration (to prevent lactic acidosis).

Review Questions

Select the ONE best answer.

1. A 10-month-old child is being evaluated for the underlying cause of a hemolytic anemia. In the diagram below, the oxygen dissociation curve for hemoglobin in his erythrocytes is compared with the curve obtained with normal red cells.



A deficiency of which enzyme is most likely to account for the hemolytic anemia in this patient?

A. Glucokinase
B. Glucose 6-P dehydrogenase
C. Pyruvate carboxylase
D. Glutathione reductase
E. Pyruvate kinase

2. A breast-fed infant begins to vomit frequently and lose weight. Several days later she is jaundiced, her liver is enlarged, and cataracts are noticed in her lenses. These symptoms are most likely caused by a deficiency of

A. galactose 1-P uridyltransferase
B. lactase
C. glucose-6-phosphatase
D. galactokinase
E. aldolase B

3. Following an early-morning run, a 29-year-old man consumes an all-American breakfast consisting of cereal, eggs, bacon, sausage, pancakes with maple syrup, doughnuts, and coffee with cream and sugar. Which of the following proteins will most likely be activated in his liver after breakfast?

    A. Cytoplasmic PEP carboxykinase
    B. Plasma membrane GLUT-4 transporter
    C. Cytoplasmic phosphofructokinase-2
    D. Mitochondrial carnitine transporter
    E. Cytoplasmic glycogen phosphorylase

Items 4 and 5

A 55-year-old alcoholic was brought to the emergency department by his friends. During their usual nightly gathering at the local bar, he had passed out and they had been unable to revive him. The physician ordered an injection of thiamine followed by overnight parenteral glucose. The next morning the patient was alert and coherent, serum thiamine was normal, and blood glucose was 73 mg/dL (4 mM). The IV line was removed and he was taken home.

4. Which of the following enzymes is thiamine-dependent and essential for glucose oxidation in the brain?

    A. Transketolase
    B. Transaldolase
    C. Succinyl-CoA thiokinase
    D. Acetyl-CoA carboxylase
    E. Pyruvate dehydrogenase

5. At the time of discharge from the hospital, which of the following proteins would have no significant physiologic activity in this patient?

    A. Malate dehydrogenase
    B. Glucokinase
    C. α-Ketoglutarate dehydrogenase
    D. GLUT 1 transporter
    E. Phosphofructokinase-1

Answers

1. **Answer: E.** A right-shift in the O_2 binding curve is indicative of abnormally elevated 2,3-BPG secondary to a defect in red cell anaerobic glycolysis. Only pyruvate kinase participates in this pathway.

2. **Answer: A.** Cataracts + liver disease in a milk-fed infant = classic galactosemia.

3. **Answer: C.** Only PFK-2 will be insulin-activated in the postprandial period.

4. **Answer: E.** Most important TPP-dependent enzymes include pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase. Transketolase is in the HMP shunt and is not strictly essential for glucose oxidation.

5. **Answer: B.** After an overnight fast (plasma glucose 73 mg/dL), the liver is producing glucose and glucokinase activity would be insignificant (high K_m, low insulin). The other proteins would be needed for aerobic glucose oxidation in the brain or for hepatic gluconeogenesis.

Citric Acid Cycle and Oxidative Phosphorylation

13

Learning Objectives

- ❑ Solve problems concerning citric acid cycle
- ❑ Explain information related to electron transport chain
- ❑ Understand concepts of oxidative phosphorylation

CITRIC ACID CYCLE

The citric acid cycle (also called Krebs cycle or tricarboxylic acid cycle), is in the mitochondria. Although oxygen is not directly required in the cycle, the pathway will not occur anaerobically because NADH and $FADH_2$ will accumulate if oxygen is not available for the electron transport chain.

The primary function of the cycle is oxidation of acetyl-CoA to carbon dioxide. The energy released from this oxidation is saved as NADH, $FADH_2$, and guanosine triphosphate (GTP). The overall result of the cycle is represented by the following reaction:

$$\begin{array}{lcl} \text{Acetyl-CoA} & \longrightarrow & 2\ CO_2 \\ 3\ NAD + FAD + GDP + P_i & & 3\ NADH + FADH_2 + GTP \end{array}$$

Notice that none of the intermediates of the citric acid cycle appear in this reaction, not as reactants or as products. This emphasizes an important (and frequently misunderstood) point about the cycle.

- It does not represent a pathway for the net conversion of acetyl-CoA to citrate, to malate, or to any other intermediate of the cycle.
- The only fate of acetyl-CoA in this pathway is its oxidation to CO_2.
- Thus, the citric acid cycle does not represent a pathway by which there can be net synthesis of glucose from acetyl-CoA.

The cycle is central to the oxidation of any fuel that yields acetyl-CoA, including glucose, fatty acids, ketone bodies, ketogenic amino acids, and alcohol. There is no hormonal control of the cycle, as activity is necessary irrespective of the fed or fasting state. Control is exerted by the energy status of the cell through allosteric activation or deactivation. Many enzymes are subject to negative feedback.

In the citric acid cycle, all the enzymes are in the matrix of the mitochondria except succinate dehydrogenase, which is in the inner membrane.

Key points:

- Isocitrate dehydrogenase, the major control enzyme, is inhibited by NADH and ATP and activated by ADP.
- α-ketoglutarate dehydrogenase, like pyruvate dehydrogenase, is a multienzyme complex. It requires thiamine, lipoic acid, CoA, FAD, and NAD. Lack of thiamine slows oxidation of acetyl-CoA in the citric acid cycle.
- Succinyl-CoA synthetase (succinate thiokinase) catalyzes a substrate-level phosphorylation of GDP to GTP.
- Succinate dehydrogenase is on the inner mitochondrial membrane, where it also functions as complex II of the electron transport chain.
- Citrate synthase condenses the incoming acetyl group with oxaloacetate to form citrate.

Note

GTP is energetically equivalent to ATP:

GTP + ADP ↔ GDP + ATP is catalyzed by nucleoside diphosphate kinase.

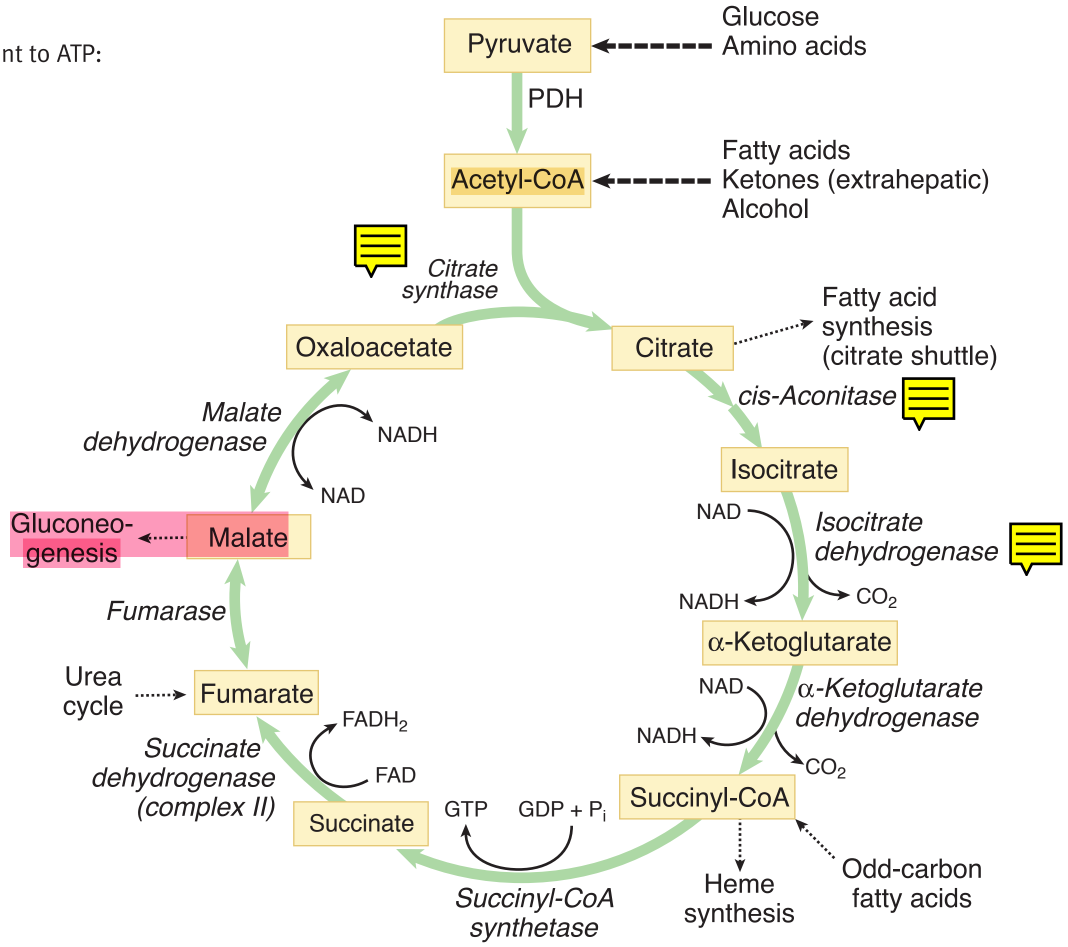


Figure I-13-1. Citric Acid Cycle

Several intermediates of the cycle may serve other functions:

- Citrate may leave the mitochondria (citrate shuttle) to deliver acetyl-CoA into the cytoplasm for fatty acid synthesis.
- Succinyl-CoA is a high-energy intermediate that can be used for heme synthesis and to activate ketone bodies in extrahepatic tissues.
- Malate can leave the mitochondria (malate shuttle) for gluconeogenesis.

When intermediates are drawn out of the citric acid cycle, the cycle slows. Therefore, when intermediates leave the cycle they must be replaced to ensure sufficient energy for the cell.

ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

The mitochondrial electron transport chain (ETC) carries out 2 reactions:

$$NADH + O_2 \longrightarrow NAD + H_2O \qquad \Delta G = -56\ \text{kcal/mol}$$

$$FADH_2 + O_2 \longrightarrow FAD + H_2O \qquad \Delta G = -42\ \text{kcal/mol}$$

Although the value of ΔG should not be memorized, it does indicate the large amount of energy released by both reactions. The electron transport chain is a device to capture this energy in a form useful for doing work.

Sources of NADH, $FADH_2$, and O_2

Many enzymes in the mitochondria—including those of the citric acid cycle and pyruvate dehydrogenase—produce NADH, all of which can be oxidized in the electron transport chain and in the process, capture energy for ATP synthesis by oxidative phosphorylation.

- If NADH is produced in the cytoplasm, the malate or α-glycerol phosphate shuttle can transfer the electrons into the mitochondria for delivery to the ETC.
- Once NADH has been oxidized, the NAD can again be used by enzymes that require it.

$FADH_2$ is produced by succinate dehydrogenase in the citric acid cycle and by the α-glycerol phosphate shuttle. Both enzymes are located in the inner membrane and can reoxidize $FADH_2$ directly by transferring electrons into the ETC. Once $FADH_2$ has been oxidized, the FAD can be made available once again for use by the enzyme.

O_2 is delivered to tissues by hemoglobin. The majority of oxygen required in a tissue is consumed in the ETC. Its function is to accept electrons at the end of the chain, and the water formed is added to the cellular water.

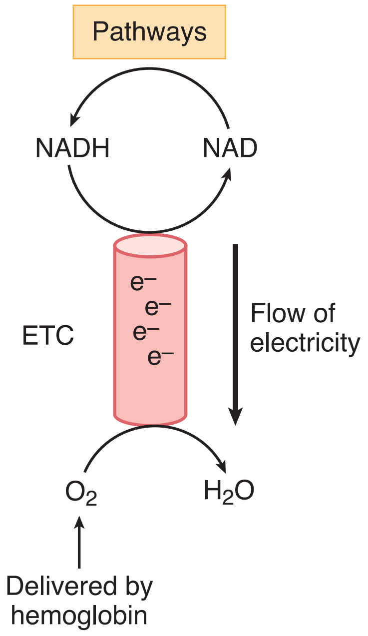


Figure I-13-2. Electron Transport Chain

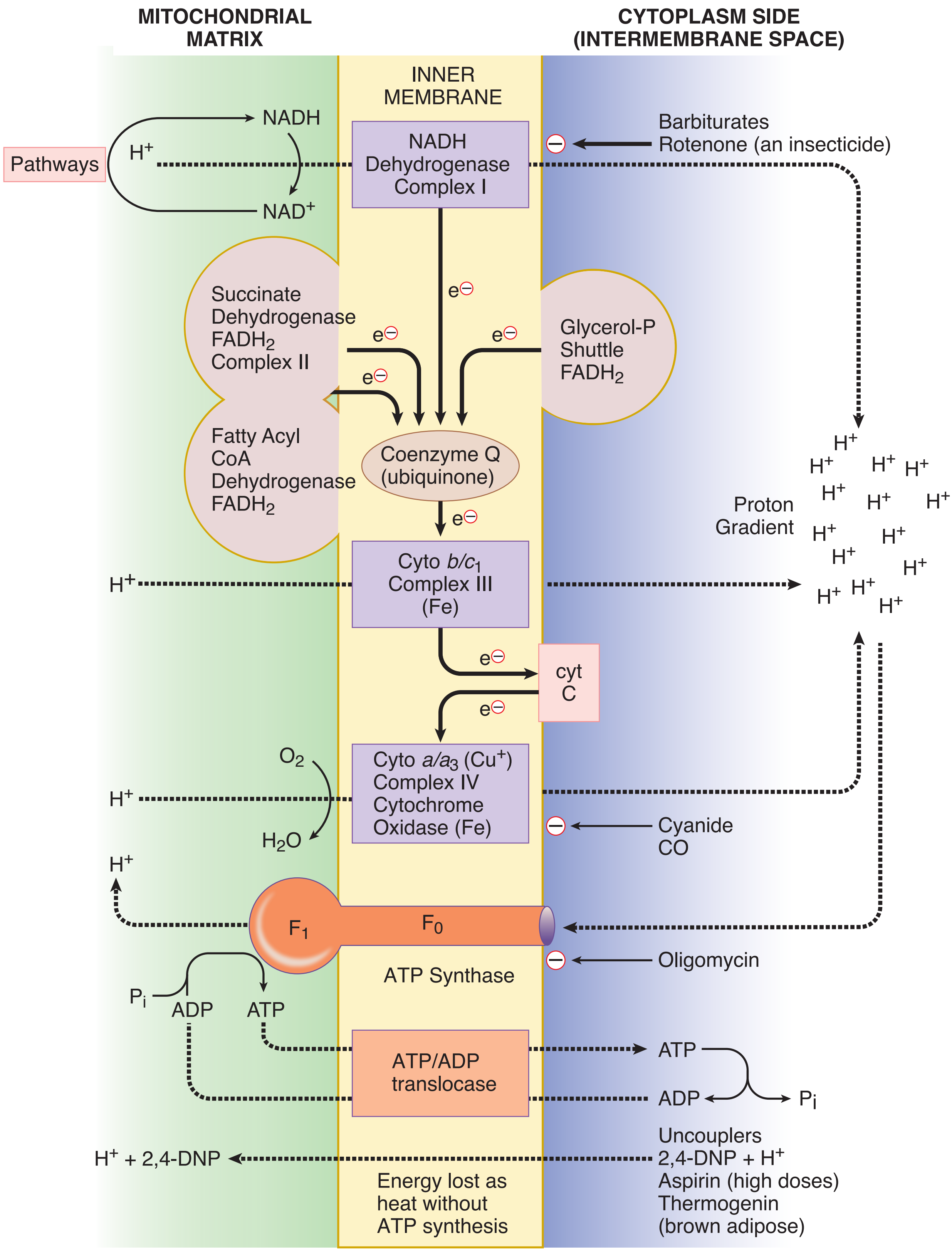


Figure I-13-3. Oxidative Phosphorylation

Capturing Chemical Energy as Electricity

The mitochondrial electron transport chain works like a chemical battery. In one location, an oxidation reaction is poised to release electrons at very high energy; in another location, a potential electron acceptor waits to be reduced. Because the 2 components are physically separated, nothing happens. Once the 2 terminals of the battery are connected by a wire, electrons flow from one compartment to the other through the wire, producing an electrical current or electricity. A light bulb or an electrical pump inserted into the circuit will run on the electricity generated. If no electrical device is in the circuit, all the energy is released as heat. The mitochondrial electron transport chain operates according to the same principle.

Electron Transport Chain

High-Yield

NADH is oxidized by NADH dehydrogenase (complex I), delivering its electrons into the chain and returning as NAD to enzymes that require it. The electrons are passed along a series of protein and lipid carriers that serve as the wire.

These include, in order:

- NADH dehydrogenase (complex I) accepts electrons from NADH
- Coenzyme Q (a lipid)
- Cytochrome b/c_1 (an Fe/heme protein; complex III)
- Cytochrome c (an Fe/heme protein)
- Cytochrome a/a_3 (a Cu/heme protein; cytochrome oxidase, complex IV) transfers electrons to oxygen

All these components are in the inner membrane of the mitochondria as shown below. Succinate dehydrogenase and the α-glycerol phosphate shuttle enzymes reoxidize their $FADH_2$ and pass electrons directly to CoQ.

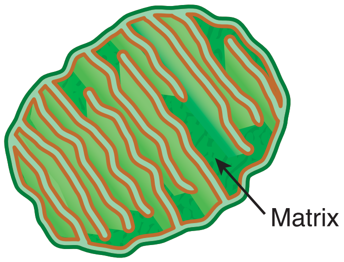


Figure I-13-4. Mitochondrion

Proton Gradient

The electricity generated by the ETC is used to run proton pumps (translocators), which drive protons from the matrix space across the inner membrane into the intermembrane space, creating a small proton (or pH) gradient. This is similar to pumping any ion, such as Na^+, across a membrane to create a gradient. The 3 major complexes I, III, and IV (NADH dehydrogenase, cytochrome b/c_1, and cytochrome a/a_3) all translocate protons in this way as the electricity passes through them.

The end result is that a proton gradient is normally maintained across the mitochondrial inner membrane. If proton channels open, the protons run back into the matrix. Such proton channels are part of the oxidative phosphorylation complex.

Oxidative Phosphorylation

High-Yield

ATP synthesis by oxidative phosphorylation uses the energy of the proton gradient and is carried out by the F_0F_1 ATP synthase complex, which spans the inner membrane. As protons flow into the mitochondria through the F_0 component, their energy is used by the F_1 component (ATP synthase) to phosphorylate ADP using P_i.

Bridge to Pathology

A genetic defect in oxidative phosphorylation is one cause of Leigh syndrome, a rare neurological disorder.

On average, when an NADH is oxidized in the ETC, sufficient energy is contributed to the proton gradient for the phosphorylation of 3 ATP by F_0F_1 ATP synthase. $FADH_2$ oxidation provides enough energy for approximately 2 ATP. These figures are referred to as the P/O ratios.

Tissue Hypoxia

High-Yield

Hypoxia deprives the ETC of sufficient oxygen, decreasing the rate of ETC and ATP production. When ATP levels fall, glycolysis increases and, in the absence of oxygen, will produce lactate (lactic acidosis). Anaerobic glycolysis is not able to meet the demand of most tissues for ATP, especially in highly aerobic tissues like nerves and cardiac muscle.

In a myocardial infarction (MI), myocytes swell as the membrane potential collapses and the cell gets leaky. Enzymes are released from the damaged tissue, and lactic acidosis contributes to protein precipitation and coagulation necrosis.

Bridge to Pathology

Ischemic Chest Pain

Patients with chest pain whose symptoms are suggestive of acute myocardial infarction (AMI) are evaluated by electrocardiogram and serial cardiac enzymes. Although myocardial-specific CK-MB has been used as an early indicator of an AMI, troponin levels are rapidly replacing it.

Troponin I and troponin T are sensitive and specific markers that appear 3–6 hours after the onset of symptoms, peak by 16 hours, and remain elevated for nearly a week. In the absence of ST-segment elevation on the EKG, elevated troponin I and troponin T are useful indicators for those at high risk for evolving myocardial infarction. LDH isozyme analysis may be helpful if a patient reports chest pain that occurred several days previously because this change ($LDH_1 > LDH_2$) peaks 2–3 days following an AMI.

Inhibitors

High-Yield

The ETC is coupled to oxidative phosphorylation so that their activities rise and fall together. Inhibitors of any step effectively inhibit the whole coupled process, resulting in:

- Decreased oxygen consumption
- Increased intracellular NADH/NAD and $FADH_2$/FAD ratios
- Decreased ATP

Important inhibitors include cyanide and carbon monoxide.

Cyanide is a deadly poison because it binds irreversibly to cytochrome a/a_3, preventing electron transfer to oxygen, and producing many of the same changes seen in tissue hypoxia. Sources of cyanide include:

- Burning polyurethane (foam stuffing in furniture and mattresses)
- Byproduct of nitroprusside (released slowly; thiosulfate can be used to destroy the cyanide)

Nitrites may be used as an antidote for cyanide poisoning if given rapidly. They convert hemoglobin to methemoglobin, which binds cyanide in the blood before reaching the tissues. Oxygen is also given, if possible.

Carbon monoxide binds to cytochrome a/a_3 but less tightly than cyanide. It also binds to hemoglobin, displacing oxygen. Symptoms include headache, nausea, tachycardia, and tachypnea. Lips and cheeks turn a cherry-red color. Respiratory depression and coma result in death if not treated by giving oxygen. Sources of carbon monoxide include:

- Propane heaters and gas grills
- Vehicle exhaust
- Tobacco smoke
- House fires
- Methylene chloride–based paint strippers

Other inhibitors include antimycin (cytochrome b/c_1), doxorubicin (CoQ), and oligomycin (F_0).

Uncouplers are chemicals that decrease the proton gradient, causing:

- Decreased ATP synthesis
- Increased oxygen consumption
- Increased oxidation of NADH

Because the rate of the ETC increases, with no ATP synthesis, energy is released as heat. Important uncouplers include 2,4-dinitrophenol (2,4-DNP) and aspirin (and other salicylates). Brown adipose tissue contains a natural uncoupling protein (UCP, formerly called thermogenin), which allows energy loss as heat to maintain a basal temperature around the kidneys, neck, breastplate, and scapulae in newborns.

Bridge to Pharmacology

Aspirin in high doses used to treat rheumatoid arthritis can result in uncoupling of oxidative phosphorylation, increased oxygen consumption, depletion of hepatic glycogen, and the pyretic effect of toxic doses of salicylate. Depending on the degree of salicylate intoxication, symptoms can vary from tinnitus to pronounced CNS and acid-base disturbance.

Recall Question

Which of the following substrates is used in heme synthesis?

A. Citrate

B. Fumarate

C. Succinate

D. Succinyl-CoA

Answer: D

Reactive Oxygen Species

High-Yield

When molecular oxygen (O_2) is partially reduced, unstable products called reactive oxygen species (ROS) are formed. These react rapidly with lipids to cause peroxidation, with proteins, and with other substrates, resulting in denaturation and precipitation in tissues. Reactive oxygen species include:

- Superoxide ($O_2^{\cdot -}$)
- Hydrogen peroxide (H_2O_2)
- Hydroxyl radical ($OH^{\cdot}$)

The polymorphonuclear neutrophil produces these substances to kill bacteria in the protective space of the phagolysosome during the oxidative burst accompanying phagocytosis. Production of these same ROS can occur at a slower rate wherever there is oxygen in high concentration. Small quantities of ROS are inevitable by-products of the electron transport chain in mitochondria. These small quantities are normally destroyed by protective enzymes such as catalase. The rate of ROS production can increase dramatically under certain conditions, such as reperfusion injury in a tissue that has been temporarily deprived of oxygen. ATP levels will be low and NADH levels high in a tissue deprived of oxygen (as in an MI). When oxygen is suddenly introduced, there is a burst of activity in the ETC, generating incompletely reduced ROS.

Defenses against ROS accumulation are particularly important in highly aerobic tissues and include superoxide dismutase and catalase. In the special case of erythrocytes, large amounts of superoxide are generated by the spontaneous dissociation of the oxygen from hemoglobin (occurrence is 0.5–3% of the total hemoglobin per day). The products are methemoglobin and superoxide. The processes that adequately detoxify the superoxide require a variety of enzymes and compounds, including superoxide dismutase, catalase, as well as glutathione peroxidase, vitamin E in membranes, and vitamin C in the cytoplasm. Low levels of any of these detoxifying substances result in hemolysis. For example, inadequate production of NADPH in glucose 6-phosphate dehydrogenase deficiency results in accumulation of the destructive hydrogen peroxide (Chapter 14).

Bridge to Medical Genetics

Mitochondrial Diseases

- Leber hereditary optic neuropathy
- Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Myoclonic epilepsy with ragged-red muscle fibers

Mutations in Mitochondrial DNA

High-Yield

The circular mitochondrial chromosome encodes 13 of the >80 proteins that comprise the major complexes of oxidative phosphorylation, as well as 22 tRNAs and 2 rRNAs. Mutations in these genes affect highly aerobic tissues (nerves, muscle), and the diseases exhibit characteristic mitochondrial pedigrees (maternal inheritance).

Key characteristics of most mitochondrial DNA (mtDNA) diseases are lactic acidosis and massive proliferation of mitochondria in muscle, resulting in ragged red fibers. Examples of mtDNA diseases are:

- Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Leber hereditary optic neuropathy
- Ragged-red muscle fiber disease

Coordinate Regulation of the Citric Acid Cycle and Oxidative Phosphorylation

The rates of oxidative phosphorylation and the citric acid cycle are closely coordinated, and are dependent mainly on the availability of O_2 and ADP.

- If O_2 is limited, the rate of oxidative phosphorylation decreases, and the concentrations of NADH and $FADH_2$ increase. The accumulation of NADH, in turn, inhibits the citric acid cycle. The coordinated regulation of these pathways is known as "respiratory control."
- If O_2 is adequate, the rate of oxidative phosphorylation depends on the availability of ADP. The concentrations of ADP and ATP are reciprocally related; an accumulation of ADP is accompanied by a decrease in ATP and the amount of energy available to the cell.
  - Therefore, ADP accumulation signals the need for ATP synthesis.
  - ADP allosterically activates isocitrate dehydrogenase, thereby increasing the rate of the citric acid cycle and the production of NADH and FADH2. Elevated levels of these reduced coenzymes, in turn, increase the rate of electron transport and ATP synthesis.

Review Questions

Select the ONE best answer.

1. During a myocardial infarction, the oxygen supply to an area of the heart is dramatically reduced, forcing the cardiac myocytes to switch to anaerobic metabolism. Under these conditions, which of the following enzymes would be activated by increasing intracellular AMP?

   A. Succinate dehydrogenase

   B. Phosphofructokinase-1

   C. Glucokinase

   D. Pyruvate dehydrogenase

   E. Lactate dehydrogenase

Items 2 and 3

A 40-year-old African American man is seen in the emergency room for a severe headache. His blood pressure is 180/110 mm Hg, and he has evidence of retinal hemorrhage. An infusion of nitroprusside is given.

2. Which of the following enzymes is affected most directly by the active metabolite of this drug?

   A. Phospholipase A2

   B. Cyclic AMP phosphodiesterase

   C. Guanylate cyclase

   D. Cyclic GMP phosphodiesterase

   E. Phospholipase C

3. When nitroprusside is given in higher than usual doses, it may be accompanied by the administration of thiosulfate to reduce potential toxic side effects. Which complex associated with electron transport or oxidative phosphorylation is most sensitive to the toxic byproduct that may accumulate with high doses of nitroprusside?

   A. NADH dehydrogenase

   B. Succinate dehydrogenase

   C. Cytochrome b/c_1

   D. Cytochrome a/a_3

   E. F_0F_1 ATP synthase

4. A patient has been exposed to a toxic compound that increases the permeability of mitochondrial membranes for protons. Which of the following events in liver cells would you expect to occur?

   A. Increased ATP levels
   B. Increased F_1F_0 ATP synthase activity
   C. Increased oxygen utilization
   D. Decreased malate-aspartate shuttle activity
   E. Decreased pyruvate dehydrogenase activity

Items 5 and 6

   A. Citrate shuttle
   B. Glycerolphosphate shuttle
   C. Malate-aspartate shuttle
   D. Carnitine shuttle
   E. Adenine nucleotide shuttle

5. Required for cholesterol and fatty acid synthesis in hepatocytes

6. Required for the hepatic conversion of pyruvate to glucose

Answers

1. **Answer: B.** Both PFK-1 and LDH participate in extrahepatic anaerobic glycolysis, but only PFK-1 is regulated by allosteric effectors.

2. **Answer: C.** Nitroprusside is metabolized to produce nitric oxide. NO, normally produced by the vascular endothelium, stimulates the cyclase in vascular smooth muscle to increase cGMP, activate protein kinase G, and cause relaxation.

3. **Answer: D.** In addition to NO, metabolism of nitroprusside also releases small quantities of cyanide, a potent and potentially lethal inhibitor of cyt a/a_3 (complex IV). Thiosulfate is a common antidote for CN poisoning.

4. **Answer: C.** The toxic agent (example, 2,4-dinitrophenol) would uncouple oxidative phosphorylation, leading to a fall in ATP levels, increased respiration, and increased substrate utilization.

5. **Answer: A.** Both fatty acids and cholesterol are synthesized from acetyl-CoA in the cytoplasm. Acetyl-CoA, which is produced in the mitochondria, is delivered to these pathways using the citrate shuttle.

6. **Answer: C.** Oxaloacetate, produced from pyruvate, exits the mitochondrion after conversion to malate.

Glycogen, Gluconeogenesis, and the Hexose Monophosphate Shunt

14

Learning Objectives

- ❑ Interpret scenarios about glycogenesis and glycogenolysis
- ❑ Know how glycogen synthesis is regulated
- ❑ Know how glycogenolysis is regulated
- ❑ Know the glycogen storage diseases
- ❑ Solve problems concerning gluconeogenesis
- ❑ Use knowledge of hexose monophosphate shunt

GLYCOGENESIS AND GLYCOGENOLYSIS

Glycogen, a branched polymer of glucose, represents a storage form of glucose. Glycogen synthesis and degradation occur primarily in liver and skeletal muscle, although other tissues such as cardiac muscle and the kidney store smaller quantities.

Glycogen is stored in the cytoplasm as single granules (skeletal muscle) or as clusters of granules (liver). The granule has a central protein core with polyglucose chains radiating outward to form a sphere.

- Glycogen granules composed entirely of linear chains have the highest **density** of glucose near the core.
- If the chains are branched, glucose **density** is highest at the periphery of the granule, allowing more rapid release of glucose on demand.

Glycogen stored in the liver is a source of glucose mobilized during hypoglycemia. Muscle glycogen is stored as an energy reserve for muscle contraction. In white (fast-twitch) muscle fibers, the glucose is converted primarily to lactate, whereas in red (slow-twitch) muscle fibers, the glucose is completely oxidized.

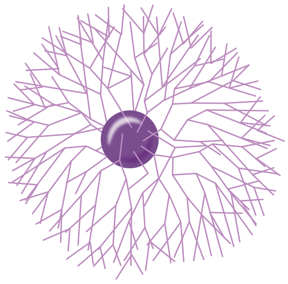


Figure I-14-1. Glycogen Granule

GLYCOGEN SYNTHESIS

Synthesis of glycogen granules begins with a core protein glycogenin. Glucose addition to a granule begins with glucose 6-phosphate, which is converted to glucose 1-phosphate and activated to UDP-glucose for addition to the glycogen chain by glycogen synthase. Glycogen synthase is the rate-limiting enzyme of glycogen synthesis.

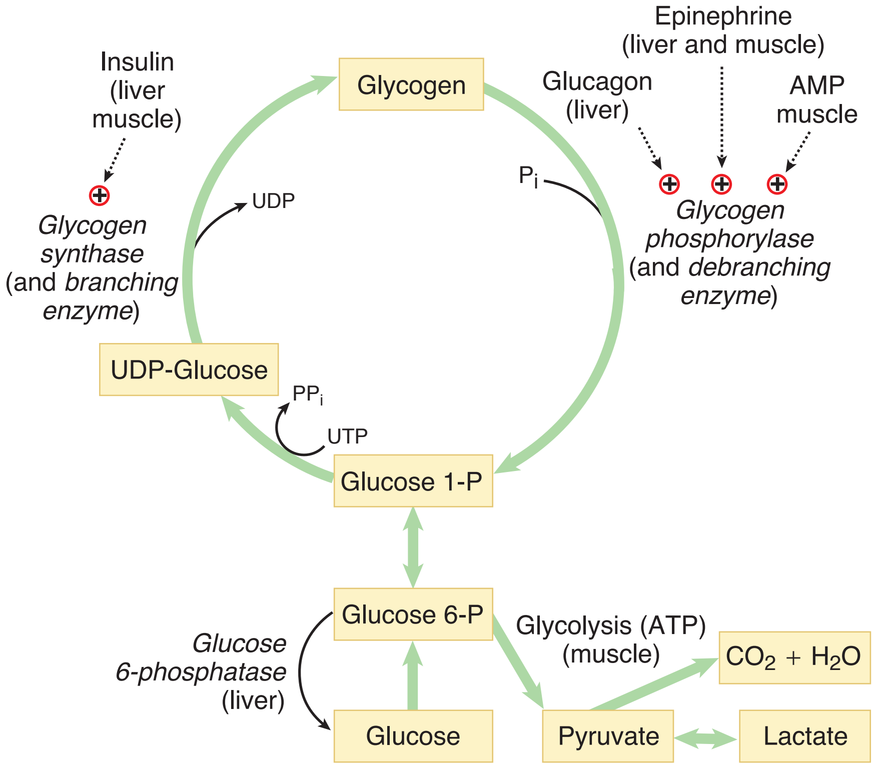


Figure I-14-2. Glycogen Metabolism

Glycogen Synthase

High-Yield

Glycogen synthase forms the α1,4 glycosidic bond found in the linear glucose chains of the granule. Note the differences in the control of glycogen synthase in the liver and skeletal muscle.

Table I-14-1. Comparison of Glycogen Synthase in Liver and Muscle

Glycogen Synthase	Liver	Skeletal Muscle
Activated by	Insulin	Insulin
Inhibited by	Glucagon, epinephrine	Epinephrine

Branching Enzyme (Glycosyl α1,4: α1,6 Transferase)

Branching enzyme is responsible for introducing α1,6-linked branches into the granule as it grows. Branching enzyme:

- Hydrolyzes one of the α1,4 bonds to release a block of oligoglucose, which is then moved and added in a slightly different location
- Forms an α1,6 bond to create a branch

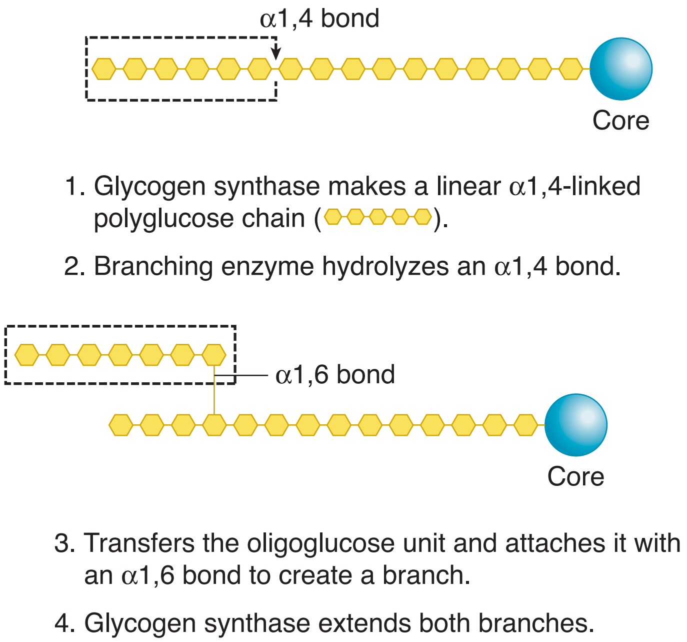


Figure I-14-3. Branching Enzyme

GLYCOGENOLYSIS

The rate-limiting enzyme of glycogenolysis is glycogen phosphorylase (in contrast to a hydrolase, a phosphorylase breaks bonds using P_i rather than H_2O). The glucose 1-phosphate formed is converted to glucose 6-phosphate by the same mutase used in glycogen synthesis.

Glycogen Phosphorylase

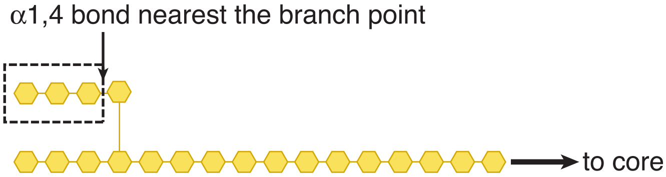
High-Yield

Glycogen phosphorylase breaks α1,4 glycosidic bonds, releasing glucose 1-phosphate from the periphery of the granule. Control of the enzyme in liver and muscle is compared below.

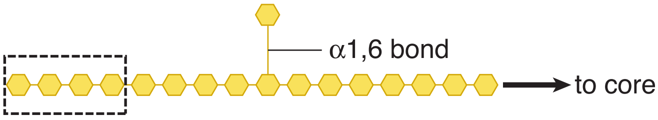
Table I-14-2. Comparison of Glycogen Phosphorylase in Liver and Muscle

Glycogen Phosphorylase	Liver	Skeletal Muscle
Activated by	Epinephrine Glucagon	Epinephrine AMP Ca^{2+} (through calmodulin)
Inhibited by	Insulin	Insulin ATP

Glycogen phosphorylase cannot break α1,6 bonds and thus stops when it nears the outermost branch points.



1. Glycogen phosphorylase releases glucose 1-P from the periphery of the granule until it encounters the first branch points.
2. Debranching enzyme hydrolyzes the α1,4 bond nearest the branch point, as shown.



3. Transfers the oligoglucose unit to the end of another chain, then
4. Hydrolyzes the α1,6 bond releasing the single glucose from the former branch.

Figure I-14-4. Debranching Enzyme

Debranching Enzyme (Glucosyl α1,4: α1,4 Transferase and α1,6 Glucosidase)

Debranching enzyme deconstructs the branches in glycogen that have been exposed by glycogen phosphorylase. This is a 2-step process. Debranching enzyme:

- Breaks an α1,4 bond adjacent to the branch point and moves the small oligoglucose chain released to the exposed end of the other chain
- Forms a new α1,4 bond
- Hydrolyzes the α1,6 bond, releasing the single residue at the branch point as free glucose; this represents the only free glucose produced directly in glycogenolysis

GENETIC DEFICIENCIES OF ENZYMES IN GLYCOGEN METABOLISM

Important genetic deficiencies are classed as glycogen storage diseases since all are characterized by an accumulation of glycogen.

Table I-14-3. Glycogen Storage Diseases

Type	Deficient Enzyme	Cardinal Clinical Features	Glycogen Structure
I: von Gierke	Glucose-6-phosphatase	Severe hypoglycemia, lactic acidosis, hepatomegaly, hyperlipidemia, hyperuricemia, short stature, doll-like facies, protruding abdomen emaciated extremities	Normal
II: Pompe	Lysosomal α1, 4-glucosidase	Cardiomegaly, muscle weakness, death by 2 years	Glycogen-like material in inclusion bodies
III: Cori	Glycogen debranching enzyme	Mild hypoglycemia, liver enlargement	Short outer branches; single glucose residue at outer branch
IV: Andersen	Branching enzyme	Infantile hypotonia, cirrhosis, death by 2 years	Very few branches, especially toward periphery
V: McArdle	Muscle glycogen phosphorylase	Muscle cramps and weakness on exercise, myoglobinuria	Normal
VI: Hers	Hepatic glycogen phosphorylase	Mild fasting hypoglycemia, hepatomegaly, cirrhosis	Normal

Glucose-6-Phosphatase Deficiency (von Gierke Disease)

High-Yield

Deficiency of hepatic glucose-6-phosphatase produces a profound fasting hypoglycemia, lactic acidosis, and hepatomegaly. Additional symptoms include:

- Glycogen deposits in the liver (glucose 6-P stimulates glycogen synthesis, and glycogenolysis is inhibited)
- Hyperuricemia predisposing to gout. Decreased P_i causes increased AMP, which is degraded to uric acid. Lactate slows uric acid excretion in the kidney.
- Hyperlipidemia with skin xanthomas
- Fatty liver

In a person with glucose-6-phosphatase deficiency, ingestion of galactose or fructose causes no increase in blood glucose, nor does administration of glucagon or epinephrine.

Lysosomal α1,4 Glucosidase Deficiency (Pompe Disease)

Pompe disease is different from the other diseases described here because the enzyme missing is not one in the normal process of glycogenolysis. The deficient enzyme normally resides in the lysosome and is responsible for digesting glycogen-like material accumulating in endosomes. In this respect, it is more similar to diseases like Tay-Sachs or even I-cell disease in which indigestible substrates accumulate in inclusion bodies.

In Pompe disease, the tissues most severely affected are those that normally have glycogen stores. With infantile onset, massive cardiomegaly is usually the cause of death, typically age <2.

Note

Glycogen storage diseases are the favorite biochemistry topic of the exam. Be sure to know:

- Clinical features
- Deficient enzyme
- Accumulating by-products

A 12-month-old girl had slowly progressing muscle weakness involving her arms and legs and developed difficulty breathing. Her liver was enlarged, and a CT revealed cardiomegaly. A muscle biopsy showed muscle degeneration with many enlarged, prominent lysosomes filled with clusters of electron-dense granules. Her parents were told that without treatment, the child's symptoms would continue to worsen and likely result in death in 1–2 years. Enzyme replacement therapy was initiated.

This child has a defect of the enzyme lysosomal α1,4 glucosidase (also called acid maltase). Coordinated glycogen breakdown with phosphorylase and debranching enzyme occurs in the cytoplasm. Although the α1,4 glucosidase participates in glycogen breakdown, the purpose of this enzyme and the reason for its location in the lysosome are unknown. Nevertheless, tissues that contain most of the body glycogen (liver and muscle) are severely affected in Pompe disease.

Recall Question

Which of the following activates the enzyme responsible for breaking alpha 1,4 glycosidic bonds?

A. ATP

B. AMP

C. Epinephrine

D. Glucagon

Answer: B

Myophosphorylase Deficiency (McArdle Disease)

High-Yield

A 25-year-old woman had a lifelong history of exercise intolerance that was often accompanied by episodes of cramping. The episodes were somewhat ameliorated by drinking sucrose-rich soft drinks immediately before exercise. The latest episode occurred during her first spin class (stationary bicycling with a resistance load) at her local bicycle shop. She initially had extreme weakness in both legs and muscle cramps and later excreted red-brown urine. In subsequent sessions, in addition to the high-sucrose drink, she reduced the load on the bicycle and was better able to tolerate the initial phase of exercise. After 10–15 minutes, she experienced a "second wind" and was able to continue her exercise successfully.

Myophosphorylase is another name for the muscle glycogen phosphorylase.

Symptoms of myophosphorylase deficiency include exercise intolerance during the initial phase of intense exercise, muscle cramping, possible myoglobinuria, and recovery (or "second wind") after 10–15 minutes of exercise.

The woman in our vignette has myophosphorylase deficiency and is unable to properly break down glycogen to glucose 6-phosphate in her muscles. Without an adequate supply of glucose, sufficient energy via glycolysis for carrying out muscle contraction cannot be obtained, explaining why the muscles are not functioning well (weakness and cramps). The situation is improved by drinking the sucrose-containing drink, which provides dietary glucose for the muscles to use.

Hepatic Glycogen Phosphorylase Deficiency (Hers Disease)

Hepatic glycogen phosphorylase deficiency is usually a mild disease because gluconeogenesis compensates for the lack of glycogenolysis. If present, hypoglycemia, hyperlipidemia, and hyperketosis are mild. Hepatomegaly and growth retardation may be present in early childhood, although hepatomegaly may improve with age.

GLUCONEOGENESIS

During fasting, the liver maintains glucose levels in blood through glycogenolysis or gluconeogenesis. These pathways are promoted by glucagon and epinephrine and inhibited by insulin. In fasting, glycogen reserves drop dramatically in the first 12 hours, during which time gluconeogenesis increases. After 24 hours, it represents the sole source of glucose.

Important substrates for gluconeogenesis are:

- Glycerol 3-phosphate (from triacylglycerol in adipose)
- Lactate (from anaerobic glycolysis)
- Gluconeogenic amino acids (protein from muscle)

Table I-14-4. Glucogenic and Ketogenic Amino Acids

Ketogenic	Ketogenic and Glucogenic	Glucogenic
Leucine Lysine	Phenylalanine Tyrosine Tryptophan Isoleucine Threonine	All others

Dietary fructose and galactose can also be converted to glucose in the liver.

In humans, it is not possible to convert acetyl-CoA to glucose. Inasmuch as most fatty acids are metabolized solely to acetyl-CoA, they are not a major source of glucose either. One minor exception is odd-number carbon fatty acids (e.g., C17), which yield a small amount of propionyl-CoA that is gluconeogenic.

Most steps in this figure represent a reversal of glycolysis, and several of these have been omitted here.

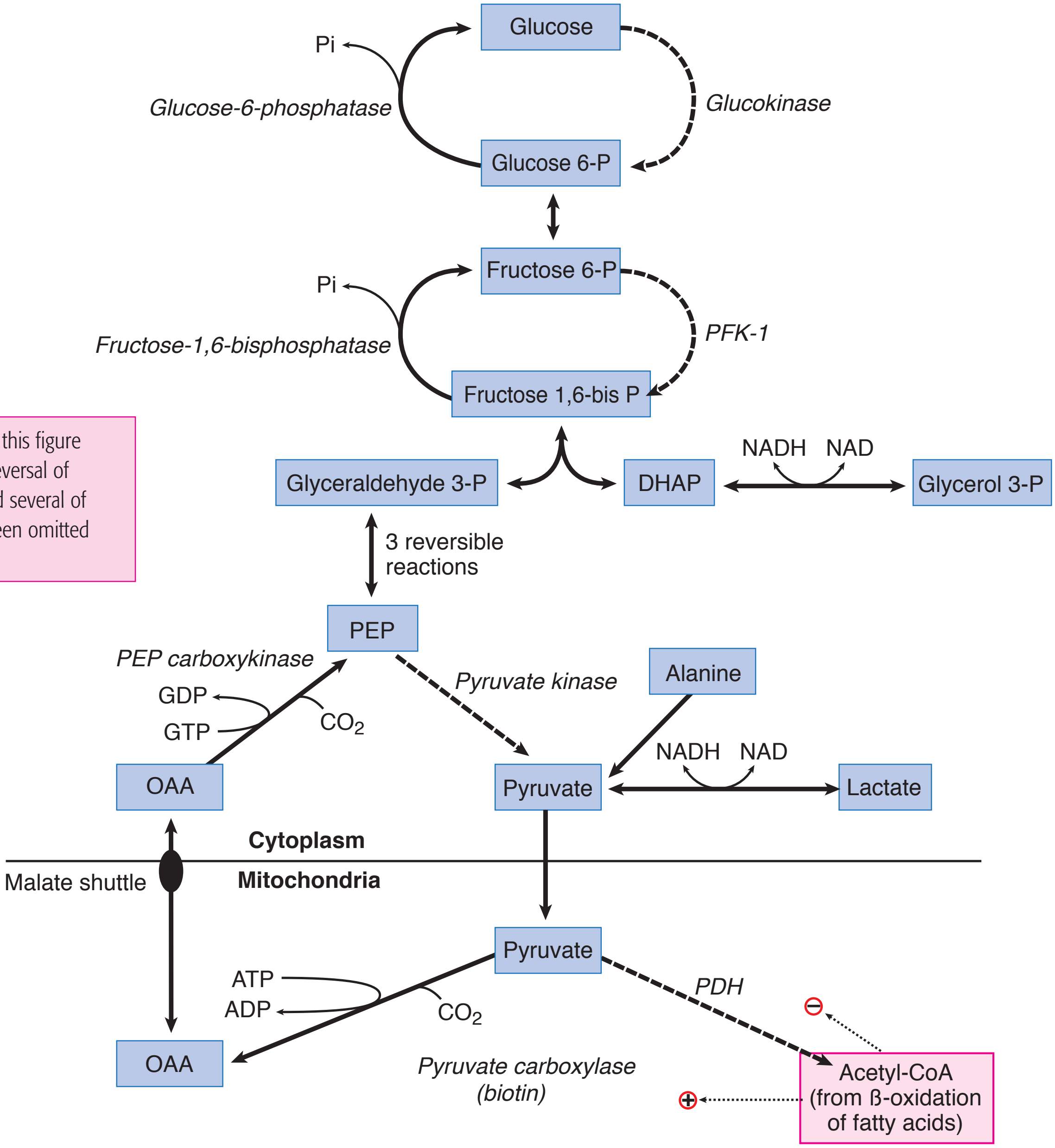


Figure I-14-5. Gluconeogenesis

In the pathway of gluconeogenesis, lactate is oxidized to pyruvate by lactate dehydrogenase. The important gluconeogenic amino acid alanine is converted to pyruvate by alanine aminotransferase (ALT or GPT). Glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate (DHAP) by glycerol 3-phosphate dehydrogenase.

The 4 important enzymes are those required to catalyze reactions that circumvent the irreversible steps:

- **Pyruvate carboxylase** is a mitochondrial enzyme requiring biotin. It is activated by acetyl-CoA (from β-oxidation). The product oxaloacetate (OAA), a citric acid cycle intermediate, cannot leave the mitochondria but is reduced to malate that can leave via the **malate shuttle**. In the cytoplasm, malate is reoxidized to OAA.
- **Phosphoenolpyruvate carboxykinase** (PEPCK) in the cytoplasm is induced by glucagon and cortisol. It converts OAA to phosphoenolpyruvate (PEP) in a reaction that requires GTP. PEP continues in the pathway to fructose 1,6-bisphosphate.
- **Fructose-1,6-bisphosphatase** in the cytoplasm is a key control point of gluconeogenesis. It hydrolyzes phosphate from fructose 1,6-bisphosphate rather than using it to generate ATP from ADP. A common pattern to note is that phosphatases oppose kinases. Fructose-1,6-bisphosphatase is activated by ATP and inhibited by AMP and fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate, produced by PFK-2, controls both gluconeogenesis and glycolysis (in the liver). Recall that PFK-2 is activated by insulin and inhibited by glucagon. Thus, glucagon will lower F 2,6-BP and stimulate gluconeogenesis, whereas insulin will increase F 2,6-BP and inhibit gluconeogenesis.
- **Glucose-6-phosphatase** is in the lumen of the endoplasmic reticulum. Glucose 6-phosphate is transported into the ER, and free glucose is transported back into the cytoplasm from which it leaves the cell. Glucose-6-phosphatase is only in the liver. The absence of glucose-6-phosphatase in skeletal muscle accounts for the fact that muscle glycogen cannot serve as a source of blood glucose (*see* Chapter 17).

Although alanine is the major gluconeogenic amino acid, 18 of the 20 (all but leucine and lysine) are also gluconeogenic. Most of these are converted by individual pathways to citric acid cycle intermediates, then to malate, following the same path from there to glucose.

It is important to note that glucose produced by hepatic gluconeogenesis does not represent an energy source for the liver. Gluconeogenesis requires expenditure of ATP that is provided by β-oxidation of fatty acids.

Therefore, hepatic gluconeogenesis is always dependent on β-oxidation of fatty acids in the liver. During hypoglycemia, adipose tissue releases these fatty acids by breaking down triglyceride.

Although the acetyl-CoA from fatty acids cannot be converted to glucose, it can be converted to ketone bodies as an alternative fuel for cells, including the brain. Chronic hypoglycemia is thus often accompanied physiologically by an increase in ketone bodies.

Note

Biotin deficiency is caused by raw egg whites (avidin) or long-term home TPN. Symptoms include:

- Alopecia
- Scaly dermatitis
- Waxy pallor
- Acidosis (mild)

Coordinate Regulation of Pyruvate Carboxylase and Pyruvate Dehydrogenase by Acetyl-CoA

The 2 major mitochondrial enzymes which use pyruvate, pyruvate carboxylase and pyruvate dehydrogenase are both regulated by acetyl-CoA. This control is important in these contexts:

- Between meals, when fatty acids are oxidized in the liver for energy, accumulating acetyl-CoA activates pyruvate carboxylase and gluconeogenesis and inhibits PDH, thus preventing conversion of lactate and alanine to acetyl-CoA.
- In the well-fed, absorptive state (insulin), accumulating acetyl-CoA is shuttled into the cytoplasm for fatty acid synthesis. OAA is necessary for this transport, and acetyl-CoA can stimulate its formation from pyruvate (see Chapter 15, Figure I-15-1).

Cori Cycle and Alanine Cycle

During fasting, lactate from red blood cells (and possibly exercising skeletal muscle) is converted in the liver to glucose that can be returned to the red blood cell or muscle. This is called the Cori cycle. The alanine cycle is a slightly different version of the Cori cycle, in which muscle releases alanine, delivering both a gluconeogenic substrate (pyruvate) and an amino group for urea synthesis.

Alcoholism and Hypoglycemia

High-Yield

Alcoholics are very susceptible to hypoglycemia. In addition to poor nutrition and the fact that alcohol is metabolized to acetate (acetyl-CoA), the high amounts of cytoplasmic NADH formed by alcohol dehydrogenase and acetaldehyde dehydrogenase interfere with gluconeogenesis. High NADH favors the formation of:

- Lactate from pyruvate
- Malate from OAA in the cytoplasm
- Glycerol 3-phosphate from DHAP

The effect is to divert important gluconeogenic substrates from entering the pathway.

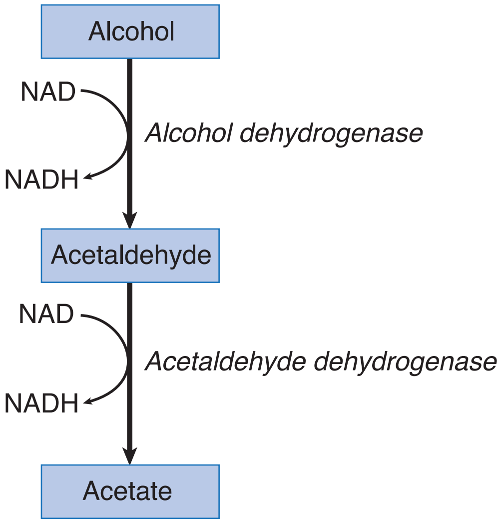


Figure I-14-6. Alcohol Metabolism

Accumulation of cytoplasmic NADH and glycerol 3-P may also contribute to lipid accumulation in alcoholic liver disease. Free fatty acids released from adipose in part enter the liver where β-oxidation is very slow (high NADH). In the presence of high glycerol 3-P, fatty acids are inappropriately stored in the liver as triglyceride.

Extreme Exercise and Alcohol Consumption

Immediately after completing a 26-mile marathon race, a healthy 24-year-old man was extremely dehydrated and thirsty. He quickly consumed a 6-pack of ice-cold beer and shortly thereafter became very weak and light-headed and nearly fainted. He complained of muscle cramping and pain.

Although the effect of alcohol is unrelated to the hormonal control of gluconeogenesis, excessive consumption of alcohol can cause severe hypoglycemia after running a marathon. In exercising muscle, lactic acid builds up in muscle due to anaerobic glycolysis, causing muscle cramping and pain. The lactate spills into blood and is converted to glucose in the liver, as part of the Cori cycle. But to carry out gluconeogenesis, NAD is required by lactate dehydrogenase to oxidize lactate to pyruvate. However, much of the available NAD is being used for ethanol metabolism and is unavailable for lactate oxidation. The result is metabolic acidosis and hypoglycemia.

Clinical Correlate

Alcohol abuse may lead to hepatic steatosis, which is fatty degeneration of liver tissue.

HEXOSE MONOPHOSPHATE SHUNT

The hexose monophosphate (HMP) shunt (pentose phosphate pathway) occurs in the cytoplasm of all cells, where it serves 2 major functions:

- NADPH production
- Source of ribose 5-phosphate for nucleotide synthesis

The first part of the HMP shunt begins with glucose 6-phosphate and ends with ribulose 5-phosphate and is irreversible. This part produces NADPH and involves the important rate-limiting enzyme glucose 6-phosphate dehydrogenase (G6PDH). G6PDH is induced by insulin, inhibited by NADPH, and activated by NADP.

Abbreviated diagram of the pathway

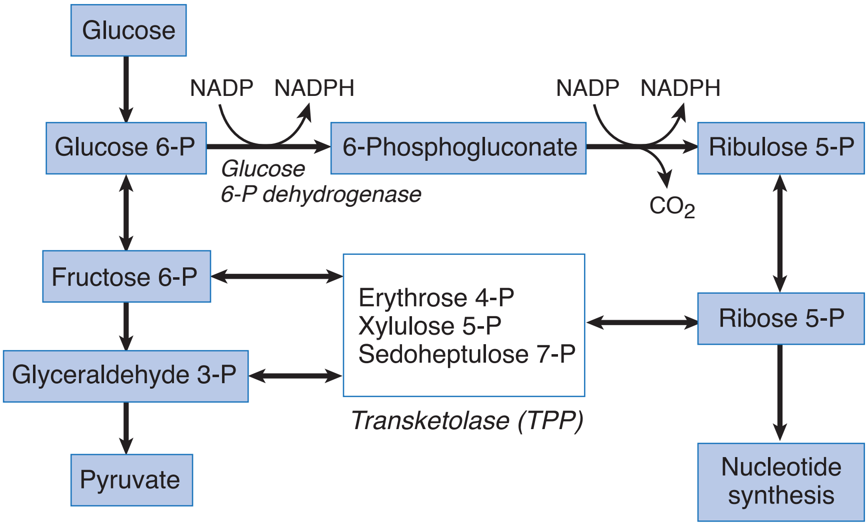


Figure I-14-7. Hexose Monophosphate Shunt

The second part of the pathway, beginning with ribulose 5-phosphate, represents a series of reversible reactions that produce an equilibrated pool of sugars for biosynthesis, including ribose 5-phosphate for nucleotide synthesis. Because fructose 6-phosphate and glyceraldehyde 3-phosphate are among the sugars produced, intermediates can feed back into glycolysis; conversely, pentoses can be made from glycolytic intermediates without going through the G6PDH reaction. Transketolase, a thiamine-requiring enzyme, is important for these interconversions; it is the only thiamine enzyme in red blood cells.

Functions of NADPH

High-Yield

Cells require NADPH for a variety of functions, including:

- Biosynthesis
- Maintenance of a supply of reduced glutathione to protect against reactive oxygen species (ROS)
- Bactericidal activity in polymorphonuclear leukocytes (PMN)

These important roles are cell-specific.

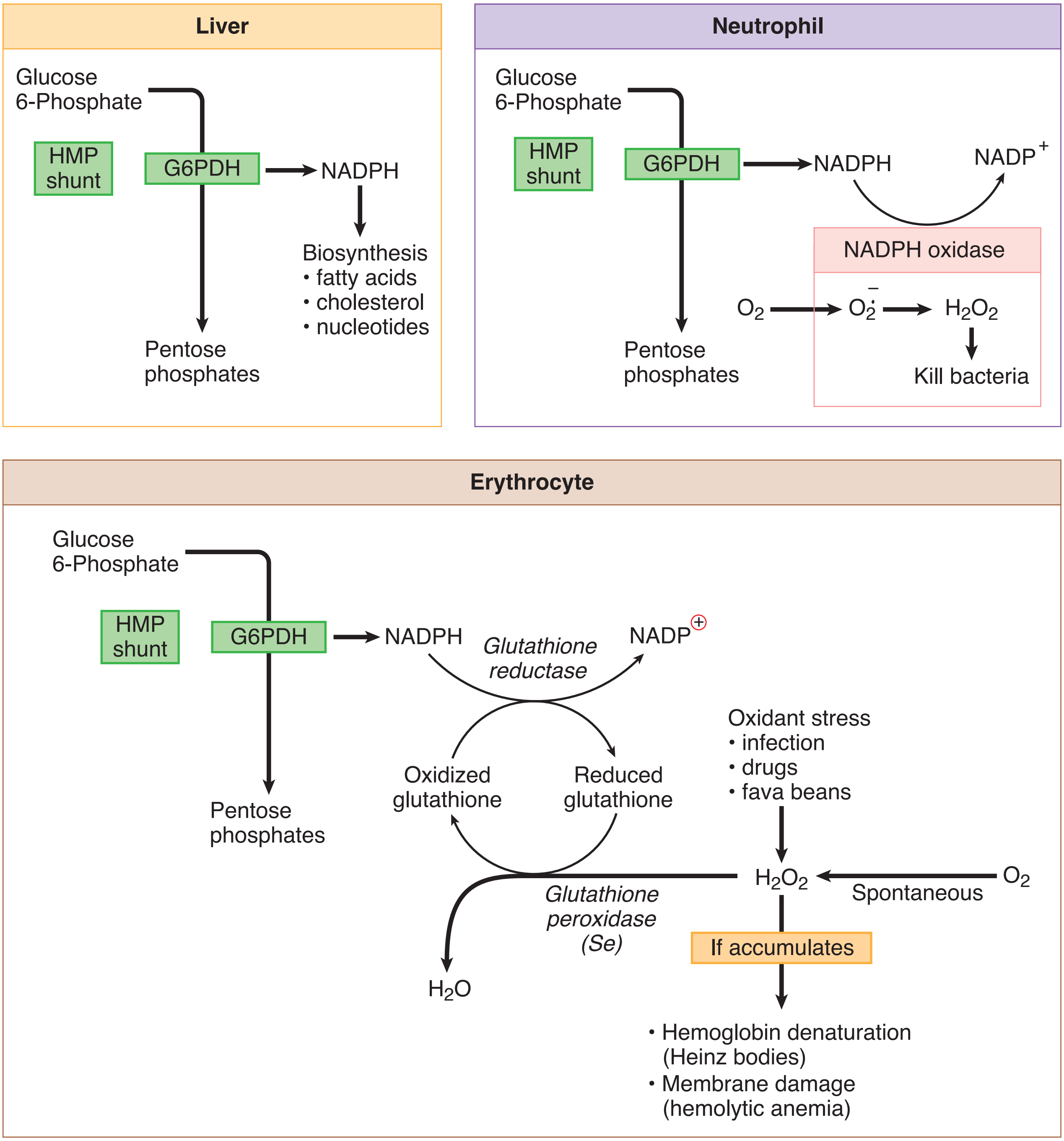


Figure I-14-8. Role of HMP Shunt in Hepatocytes, Phagocytes, and Erythrocytes

Glucose 6-Phosphate Dehydrogenase Deficiency

High-Yield

Deficiency of G6PDH may result in hemolytic anemia and, in rare cases, symptoms resembling chronic granulomatous disease (CGD). The disease shows significant allelic heterogeneity (>400 different mutations in the G6PDH gene are known). The disease is X-linked recessive.

- Major symptom is an acute episodic or chronic hemolysis (rare)
- Female heterozygous for G6PDH deficiency have increased resistance to malaria; consequently, the deficiency is often seen in families where malaria is endemic

Because red blood cells contain a large amount of oxygen, they are prone to spontaneously generate ROS that damage protein and lipid in the cell. In the presence of ROS, hemoglobin may precipitate (Heinz bodies) and membrane lipids may undergo peroxidation, weakening the membrane and causing hemolysis. As peroxides form, they are rapidly destroyed by the glutathione peroxidase/glutathione reductase system in the red blood cell, thus avoiding these complications. NADPH required by glutathione reductase is supplied by the HMP shunt in the erythrocyte.

Persons with mutations that partially destroy G6PDH activity may develop an acute, episodic hemolysis. Certain mutations affect the stability of G6PDH, and, because erythrocytes cannot synthesize proteins, the enzyme is gradually lost over time and older red blood cells lyse. This process is accelerated by certain drugs and, in a subset of patients, ingestion of fava beans. In the United States, the most likely cause of a hemolytic episode in these patients is overwhelming infection, often pneumonia (viral and bacterial) or infectious hepatitis.

In rare instances, a mutation may decrease the activity of G6PDH sufficiently to cause chronic nonspherocytic hemolytic anemia. Symptoms of CGD may also develop if there is insufficient activity of G6PDH (<5% of normal) in the PMN to generate NADPH for the NADPH oxidase bactericidal system.

Clinical Correlate

Broad beans (or fava beans) are common to diets in Mediterranean countries (Greece, Italy, Spain, Portugal, Turkey). Their ingestion may cause severe hemolysis in G6PDH individuals (**favism**). Symptoms include pallor, hemoglobinuria, jaundice, and severe anemia 24–48 hours after ingestion of the beans.

Clinical Correlate

CGD is typically caused by genetic deficiency of NADPH oxidase in the PMN. Patients are susceptible to infection by catalase-positive organisms such as *S. aureus, Klebsiella, E. coli, Candida,* and *Aspergillus*. A negative nitroblue tetrazolium test helps to confirm the diagnosis.

Bridge to Microbiology

Many parasites such as *Plasmodium* are deficient in antioxidant mechanisms, making them particularly susceptible to oxygen radicals. In G6PDH deficiency, the ability of erythrocytes to detoxify oxygen radicals is impaired. Ironically, the accumulation of the radicals in erythrocytes in G6PDH deficiency gives protection against malaria.

Review Questions

Select the ONE best answer.

1. A liver biopsy is done on a child with hepatomegaly and mild fasting hypoglycemia. Hepatocytes show accumulation of glycogen granules with single glucose residues remaining at the branch points near the periphery of the granule. The most likely genetic defect is in the gene encoding a(n):

   A. α-1,4 phosphorylase

   B. α-1,4:α-1,4 transferase

   C. phosphoglucomutase

   D. α-1,6 glucosidase

   E. lysosomal α-1,4 glucosidase

2. When fatty acid β-oxidation predominates in the liver, mitochondrial pyruvate is most likely to be

   A. carboxylated to phosphoenolpyruvate for entry into gluconeogenesis

   B. oxidatively decarboxylated to acetyl CoA for entry into ketogenesis

   C. reduced to lactate for entry into gluconeogenesis

   D. oxidatively decarboxylated to acetyl CoA for oxidation in Krebs cycle

   E. carboxylated to oxaloacetate for entry into gluconeogenesis

Items 3 and 4

A 44-year-old man from Limpopo Province in South Africa, living in the United States and receiving antibiotic therapy for a urinary tract infection, has a self-limiting episode of hemolysis, back pain, and jaundice. The peripheral blood smear reveals a nonspherocytic, normocytic anemia, and Heinz bodies are seen in some of his erythrocytes.

3. Which of the following genetic deficiencies is most likely related to his hemolytic episode?

   A. Homocysteine methyltransferase

   B. Pyruvate kinase

   C. Dihydrofolate reductase

   D. Ferrochelatase

   E. Glucose 6-phosphate dehydrogenase

4. Which of the following sets of lab results would most likely have been obtained for this patient?

	Direct Bilirubin	Indirect Bilirubin	Urinary Bilirubin
A.	Increased	Increased	Absent
B.	Increased	Increased	Present
C.	Normal	Increased	Absent
D.	Normal	Decreased	Present
E.	Increased	Decreased	Present

Answers

1. **Answer: D.** This activity of the debranching enzyme removes 1,6-linked glucose residues from the branch points during glycogenolysis.

2. **Answer: E.** Hepatic fatty acid oxidation generates energy in the postabsorptive period when pyruvate is being converted to OAA for glucose biosynthesis.

3. **Answer: E.** Only option E is consistent with the constellation of clinical findings presented. Major clue is the positive Heinz body preparation.

4. **Answer: C.** Only choice C is characteristic of hemolytic jaundice; indirect hyperbilirubinemia with no spillover of the water-insoluble unconjugated form into the urine.

Lipid Synthesis and Storage 15

Learning Objectives

- ❏ Answer questions about fatty acid nomenclature
- ❏ Understand lipid digestion
- ❏ Answer questions about fatty acid biosynthesis
- ❏ Demonstrate understanding of lipoprotein metabolism
- ❏ Explain information related to hyperlipidemias
- ❏ Use knowledge of cholesterol metabolism

FATTY ACID NOMENCLATURE

Fatty acids are long-chain carboxylic acids. The carboxyl carbon is number 1, and carbon number 2 is referred to as the α carbon. When designating a fatty acid, the number of carbons is given along with the number of double bonds (carbons:double bonds).

Palmitic acid (palmitate) is the primary end-product of fatty acid synthesis.

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO^-$

Palmitate C16:0 or 16:0

Saturated fatty acids have no double bonds.

Unsaturated fatty acids have ≥1 double bonds. Humans can synthesize only a few of the unsaturated fatty acids; the rest come from essential fatty acids in the diet that are transported as triglycerides from the intestine in chylomicrons.

Linolenic acid and **linoleic acid** are 2 important essential fatty acids. These polyunsaturated fatty acids—as well as other acids formed from them—are important in membrane phospholipids to maintain normal fluidity of cell membranes essential for many functions.

The omega (ω) numbering system is used for unsaturated fatty acids. The ω-family describes the position of the last double bond relative to the end of the chain. The omega designation identifies the major precursor fatty acid, e.g., arachidonic acid is formed from linoleic acid (ω-6 family). Arachidonic acid is itself an important precursor for prostaglandins, thromboxanes, and leukotrienes.

Linoleic	C18:2 (9,12) or $18^{\Delta 9,12}$	ω-6 family (18 – 12 = 6)
Linolenic	C18:3 (9,12,15) or $18^{\Delta 9,12,15}$	ω-3 family
Arachidonic	C20:4 (5,8,11,14) or $20^{\Delta 5,8,11,14}$	ω-6 family

Clinical Correlate

Omega-3 fatty acids in the diet are correlated with a decreased risk of cardiovascular disease. These appear to replace some of the arachidonic acid (an omega-6 fatty acid) in platelet membranes and may lower the production of thromboxane and the tendency of the platelets to aggregate.

High omega-3 fatty acids have also been associated with a decrease in serum triglycerides; they are high in some cold-water fish (salmon, tuna, herring), nuts (walnuts), and seeds (flaxseed).

Double bonds in fatty acids are in the *cis*- configuration. *Trans*- double bonds are unnatural and predominate in fatty acids found in margarine and other foods where partial hydrogenation of vegetable oils is used in their preparation. Compared with liquid oils, these partial hydrogenated fatty acids are conveniently solid at cool temperatures. When incorporated into phospholipids that constitute membranes, *trans*-fatty acids decrease membrane fluidity, similar to saturated fatty acids that are found in butter fat and other foods. *Trans*-fatty acids, as well as saturated fatty acids, are associated with increased risk of atherosclerosis.

Activation of Fatty Acids

When fatty acids are used in metabolism, they are first activated by attaching coenzyme A (CoA); fatty acyl CoA synthetase catalyzes this activation step. The product is generically referred to as a fatty acyl CoA or sometimes just acyl CoA. Specific examples would be acetyl CoA with a 2-carbon acyl group, or palmitoyl CoA with a 16-carbon acyl group.

Fatty acid + CoA + ATP → Fatty acyl CoA + AMP + PP_i

LIPID DIGESTION

Typical high-fat meals contain gram-level amounts of triglycerides and milligram-level amounts of cholesterol and cholesterol esters.

- Upon entry into the intestinal lumen, bile is secreted by the liver to emulsify the lipid contents.
- The pancreas secretes pancreatic lipase, colipase, and cholesterol esterase which degrade the lipids to 2-monoglyceride, fatty acids, and cholesterol. These lipids are absorbed and re-esterified to tryglycerides and cholesterol esters and packaged, along with apoprotein B-48 and other lipids (e.g., fat-soluble vitamins), into chylomicrons.
- Normally, there is very little lipid loss in stools. Defects in lipid digestion result in steatorrhea, in which there is an excessive amount of lipids in stool (fatty stools).

FATTY ACID BIOSYNTHESIS

Excess dietary glucose can be converted to fatty acids in the liver and subsequently sent to the adipose tissue for storage. Adipose tissue synthesizes smaller quantities of fatty acids. The pathway is shown in Figure I-15-1. Insulin promotes many steps in the conversion of glucose to acetyl CoA in the liver:

- Glucokinase (induced)
- PFK-2/PFK-1 (PFK-2 dephosphorylated)
- Pyruvate dehydrogenase (dephosphorylated)

Both of the major enzymes of fatty acid synthesis are also affected by insulin:

- Acetyl CoA carboxylase (dephosphorylated, activated)
- Fatty acid synthase (induced)

Citrate Shuttle and Malic Enzyme

The citrate shuttle transports acetyl CoA groups from the mitochondria to the cytoplasm for fatty acid synthesis. Acetyl CoA combines with oxaloacetate in the mitochondria to form citrate, but rather than continuing in the citric acid cycle, citrate is transported into the cytoplasm. Factors that indirectly promote this process include insulin and high-energy status.

In the cytoplasm, citrate lyase splits citrate back into acetyl CoA and oxaloacetate. The oxaloacetate returns to the mitochondria to transport additional acetyl CoA. This process is shown below and includes the important malic enzyme. This reaction represents an additional source of cytoplasmic NADPH in liver and adipose tissue, supplementing that from the HMP shunt.

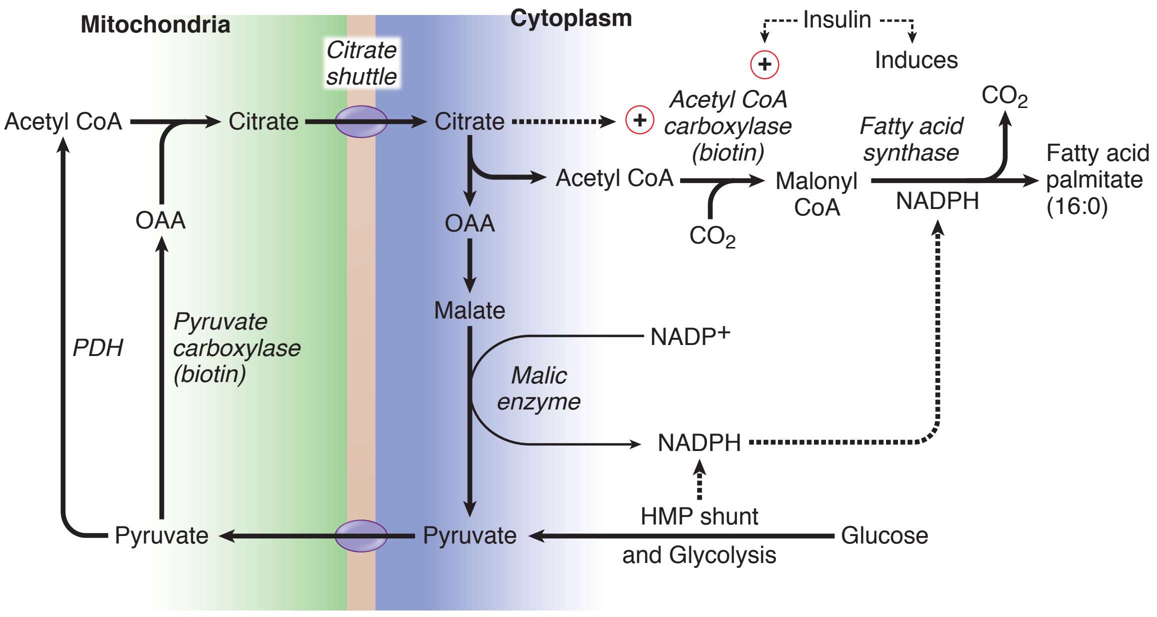


Figure I-15-1. Synthesis of Palmitate from Glucose

Acetyl CoA Carboxylase

High-Yield

Acetyl CoA is activated in the cytoplasm for incorporation into fatty acids by acetyl CoA carboxylase, the rate-limiting enzyme of fatty acid biosynthesis. Acetyl CoA carboxylase requires biotin, ATP, and CO_2. Controls include:

- Activation by insulin (dephosphorylated)
- Activation by citrate

The CO_2 added to form malonyl CoA is never incorporated into the fatty acid because it is removed by fatty acid synthase during the addition of the acetyl group to the fatty acid.

Fatty Acid Synthase

Fatty acid synthase is more appropriately called palmitate synthase because palmitate is the only fatty acid that humans can synthesize *de novo*. This enzyme is a large, multienzyme complex in the cytoplasm that is rapidly induced in the

liver after a meal by high carbohydrate and concomitantly rising insulin levels. It contains an acyl carrier protein (ACP) that requires the vitamin pantothenic acid. Although malonyl CoA is the substrate used by fatty acid synthase, only the carbons from the acetyl CoA portion are actually incorporated into the fatty acid produced. Therefore, the fatty acid is derived entirely from acetyl CoA.

NADPH is required to reduce the acetyl groups added to the fatty acid. Eight acetyl CoA groups are required to produce palmitate (16:0).

Fatty acyl CoA may be elongated and desaturated (to a limited extent in humans) using enzymes associated with the smooth endoplasmic reticulum (SER). Cytochrome b_5 is involved in the desaturation reactions. These enzymes cannot introduce double bonds past position 9 in the fatty acid.

TRIGLYCERIDE (TRIACYLGLYCEROL) SYNTHESIS

Triglycerides

Triglycerides, the storage form of fatty acids, are formed by attaching 3 fatty acids (as fatty acyl CoA) to glycerol. Triglyceride formation from fatty acids and glycerol 3-phosphate occurs primarily in liver and adipose tissue.

The liver sends triglycerides to adipose tissue packaged as very low-density lipoproteins. A small amount of triglyceride may be stored in the liver. Accumulation of significant triglyceride in tissues other than adipose tissue usually indicates a pathologic state.

There are **2 sources of glycerol 3-P** for triglyceride synthesis:

- Reduction of dihydroxyacetone phosphate (DHAP) from glycolysis by glycerol 3-P dehydrogenase, an enzyme in both adipose tissue and liver
- Phosphorylation of free glycerol by glycerol kinase, an enzyme found in liver but not in adipose tissue

Glycerol kinase allows the liver to recycle the glycerol released during VLDL metabolism (insulin) back into new triglyceride synthesis. During fasting (glucagon), this same enzyme allows the liver to trap glycerol released into the blood from lipolysis in adipose tissue for subsequent conversion to glucose.

Adipose tissue lacks glycerol kinase and is strictly dependent on glucose uptake to produce DHAP for triglyceride synthesis. In adipose tissue, the GLUT 4 transporter is stimulated by insulin, ensuring a good supply of DHAP for triglyceride synthesis.

Bridge to Pathology

Chronic alcohol use can interfere with lipid metabolism in the liver, leading to steatosis, or fatty degeneration of the liver parenchyma.

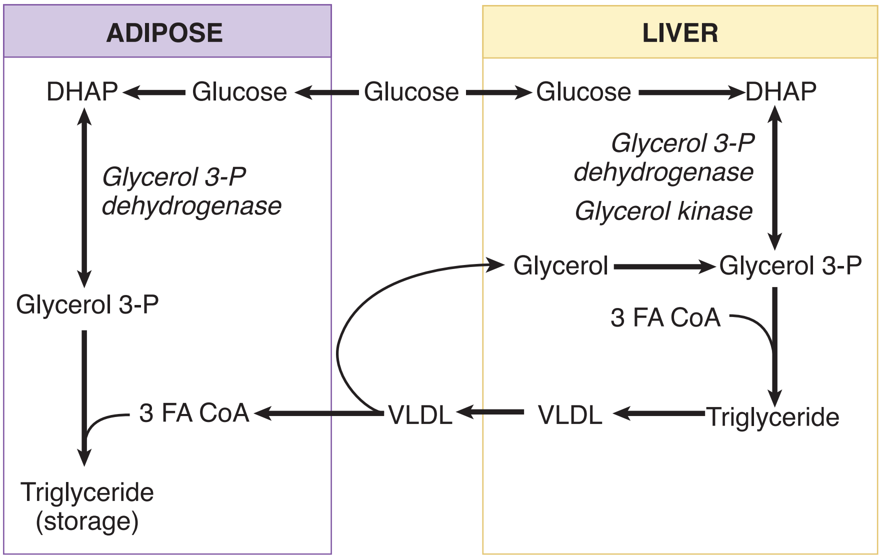


Figure I-15-2. Glycerol 3-P Dehydrogenase and Glycerol Kinase in Triglyceride Synthesis and Storage

Glycerophospholipids

High-Yield

Glycerophospholipids are used for membrane synthesis and for producing a hydrophilic surface layer on lipoproteins such as VLDL. In cell membranes, they also serve as a reservoir of second messengers such as diacylglycerol, inositol 1,4,5-triphosphate, and arachidonic acid.

The structure of glycerophospholipids is similar to that of triglycerides, except that the last fatty acid is replaced by phosphate and a water-soluble group such as choline (phosphatidylcholine, lecithin) or inositol (phosphatidylinositol).

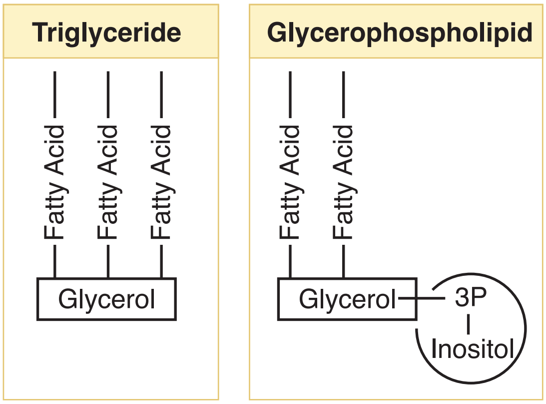


Figure I-15-3. Triglycerides and Glycerophospholipids

LIPOPROTEIN METABOLISM

Cholesterol Digestion

Triglycerides and cholesterol are transported in the blood as lipoproteins. Lipoproteins are named according to their density, which increases with the percentage of protein in the particle.

From **least dense** to **most dense**:

Chylomicrons < VLDL < IDL (intermediate-density lipoproteins)
< LDL (low-density lipoproteins) < HDL (high-density lipoproteins)

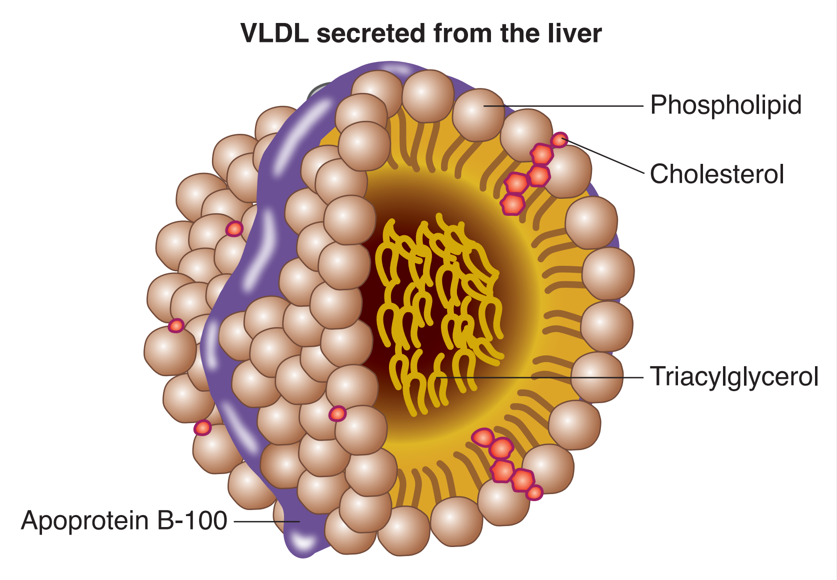


Figure I-15-4. Lipoprotein Structure

Lipoprotein and Apoprotein Classes

High-Yield

Table I-15-1. Classes of Lipoproteins and Important Apoproteins

Lipoprotein	Functions	Apoproteins	Functions
Chylomicrons	Transport dietary triglyceride and cholesterol from intestine to tissues	apoB-48 apoC-II apoE	Secreted by intestine Activates lipoprotein lipase Uptake of remnants by the liver
VLDL	Transports triglyceride from liver to tissues	apoB-100 apoC-II apoE	Secreted by liver Activates lipoprotein lipase Uptake of remnants (IDL) by liver
IDL (VLDL remnants)	Picks up cholesterol from HDL to become LDL Picked up by liver	apoE apoB-100	Uptake by liver
LDL	Delivers cholesterol into cells	apoB-100	Uptake by liver and other tissues via LDL receptor (apoB-100 receptor)
HDL	Picks up cholesterol accumulating in blood vessels Delivers cholesterol to liver and steroidogenic tissues via scavenger receptor (SR-B1) Shuttles apoC-II and apoE in blood	apoA-1	Activates lecithin cholesterol acyltransferase (LCAT) to produce cholesterol esters

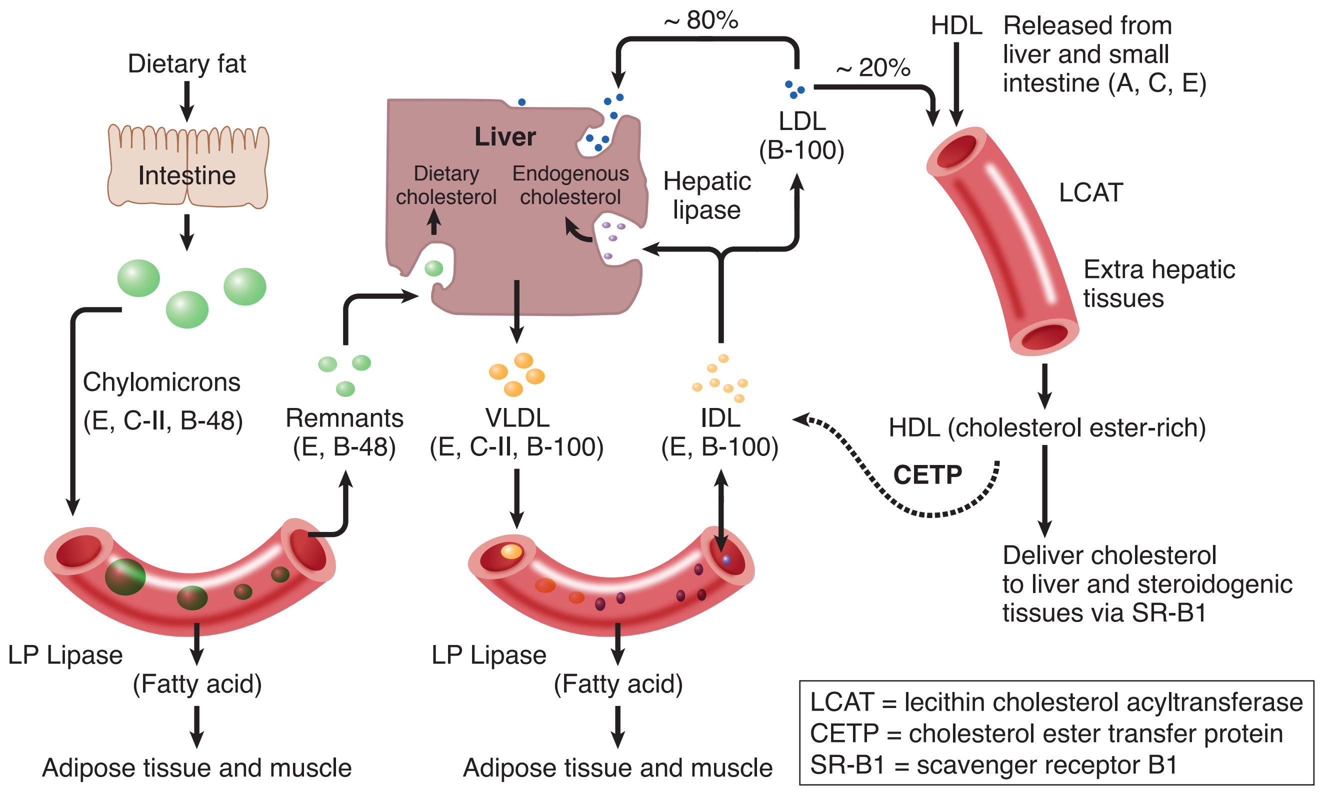


Figure I-15-5. Lipoprotein Metabolism

Chylomicrons, VLDL, and IDL

High-Yield

Chylomicrons and VLDL are primarily triglyceride particles, although they each have small quantities of cholesterol esters.

- Chylomicrons transport dietary triglyceride to adipose tissue and muscle
- VLDL transport triglyceride synthesized in the liver to these same tissues

Both chylomicrons and VLDL have apoC-II, apoE, and apoB (apoB-48 on chylomicrons and apoB-100 on VLDL).

Lipoprotein (LPLase) is required for the metabolism of both chylomicrons and VLDL. This enzyme is induced by insulin and transported to the luminal surface of capillary endothelium, where it is in direct contact with the blood. Lipoprotein lipase hydrolyzes the fatty acids from triglycerides carried by chylomicrons and VLDL and is activated by apoC-II.

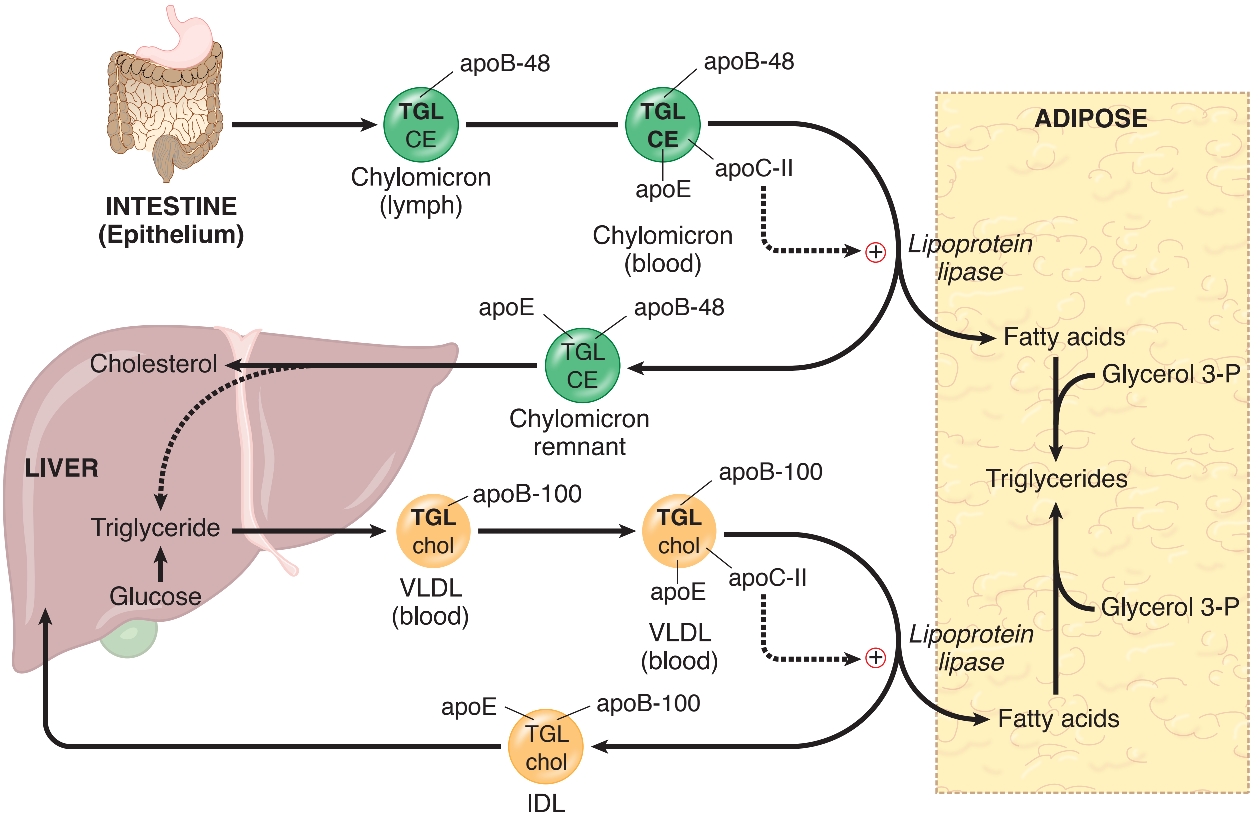


Figure I-15-6. Chylomicron and VLDL Metabolism

Chylomicrons

Chylomicrons are assembled from dietary triglycerides (containing predominantly the longer chain fatty acids, including the essential fatty acids), cholesterol esters, and the 4 lipid-soluble vitamins. The core lipid is surrounded by phospholipids similar to those found in cell membranes, which increase the solubility of chylomicrons in lymph and blood. ApoB-48 is attached and required for release from the epithelial cells into the lymphatics.

Chylomicrons leave the lymph and enter the peripheral blood, where the thoracic duct joins the left subclavian vein, thus initially bypassing the liver. After a high-fat meal, chylomicrons cause serum to become turbid or milky. While in the blood, chylomicrons acquire apoC-II and apoE from HDL particles.

In capillaries of adipose tissue (and muscle), apoC-II activates lipoprotein lipase, the fatty acids released enter the tissue for storage, and the glycerol is retrieved by the liver, which has glycerol kinase. The chylomicron remnant is picked up by hepatocytes through the apoE receptor; thus, dietary cholesterol, as well as any remaining triglyceride, is released in the hepatocyte.

VLDL

The metabolism of VLDL is very similar to that of chylomicrons, the major difference being that VLDL are assembled in hepatocytes to transport triglyceride containing fatty acids newly synthesized from excess glucose, or retrieved from the chylomicron remnants, to adipose tissue and muscle. ApoB-100 is added in the hepatocytes to mediate release into the blood. Like chylomicrons, VLDL acquire apoC-II and apoE from HDL in the blood and are metabolized by lipoprotein lipase in adipose tissue and muscle.

VLDL remnants (IDL)

After triglyceride is removed from the VLDL, the resulting particle is called a VLDL remnant or an IDL. A portion of the IDLs is picked up by hepatocytes through their apoE receptor, but some of the IDLs remain in the blood, where they are further metabolized. These IDLs are transition particles between triglyceride and cholesterol transport. In the blood, they can acquire cholesterol esters transferred from HDL particles and thus become converted into LDLs.

LDL and HDL

High-Yield

Although both LDL and HDL are primarily cholesterol particles, most of the cholesterol measured in the blood is associated with **LDL**. The normal role of LDL is to deliver cholesterol to tissues for biosynthesis. When a cell is repairing membrane or dividing, the cholesterol is required for membrane synthesis. Bile acids and salts are made from cholesterol in the liver, and many other tissues require some cholesterol for steroid synthesis. About 80% of LDL are picked up by hepatocytes, the remainder by peripheral tissues.

ApoB-100 is the only apoprotein on LDL, and endocytosis of LDL is mediated by apoB-100 receptors (LDL receptors) clustered in areas of cell membranes lined with the protein clathrin.

The liver has multiple pathways for acquiring cholesterol, including:

- *De novo* synthesis
- Endocytosis of LDL
- Transfer of cholesterol from HDL via the SR-B1 receptor
- Endocytosis of chylomicron remnants with residual dietary cholesterol

Increased cholesterol in the hepatocytes inhibits further accumulation by repressing the expression of the genes for HMG-CoA reductase, the LDL receptor, and the SR-B1 receptor.

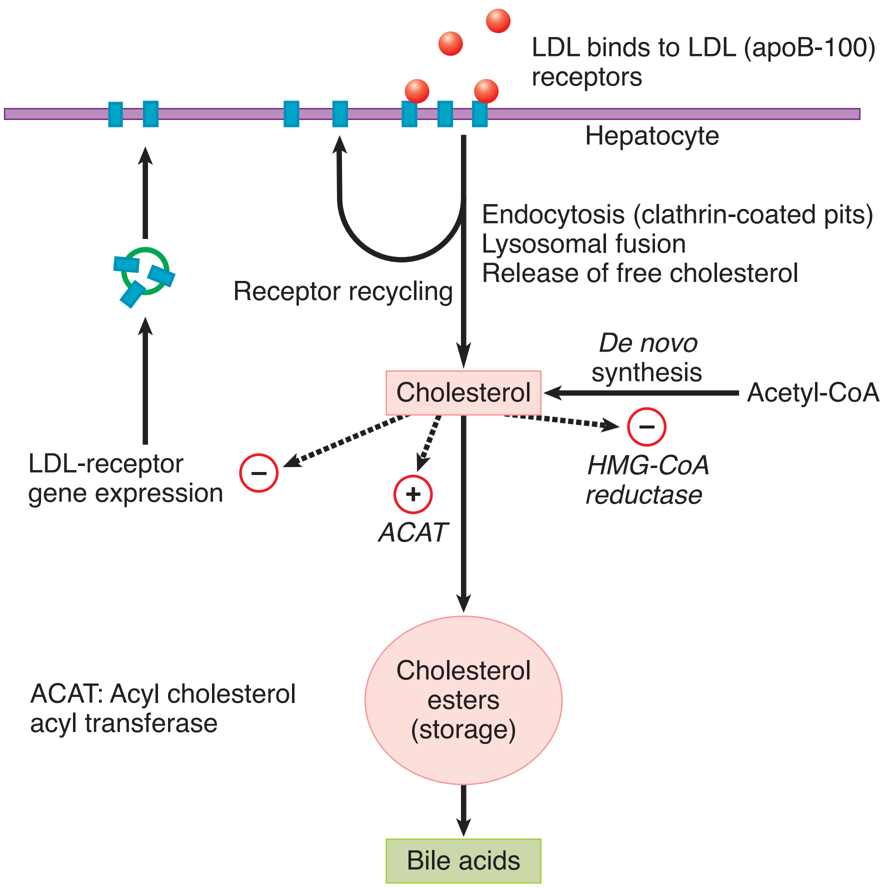


Figure I-15-7. Regulation of Cholesterol Level in Hepatocytes

Endocytosis involves:

- Formation of a coated pit, which further invaginates to become an endosome
- Fusion of the endosome with a lysosome, accompanied by acidification and activation of lysosomal enzymes
- Release of LDL from the LDL receptor

The receptor may recycle to the surface, the LDL is degraded, and cholesterol is released into the cell. Expression of the gene for LDL receptors (apoB-100 receptor) is regulated by the cholesterol level within the cell. High cholesterol decreases expression of this gene as well as the gene for HMG-CoA reductase, the rate limiting enzyme of *de novo* cholesterol synthesis.

HDL

HDL is synthesized in the liver and intestines and released as dense, protein-rich particles into the blood. The particles contain apoA-1 used for cholesterol recovery from fatty streaks in the blood vessels. HDL also carry apoE and apoC-II, but those apoproteins are primarily to donate temporarily to chylomicrons and VLDL.

- Lecithin–cholesterol acyltransferase (LCAT) (or PCAT, phosphatidylcholine–cholesterol acyltransferase) is an enzyme in the blood that is activated by apoA-1 on HDL.
  - LCAT adds a fatty acid to cholesterol, producing cholesterol esters, which dissolve in the core of the HDL.
  - This allows HDL to transport cholesterol from the periphery to the liver.
- HDL cholesterol esters picked up in the periphery can be distributed to other lipoprotein particles such as VLDL remnants (IDL), converting them to LDL. The **cholesterol ester transfer protein (CETP)** facilitates this transfer.
- HDL cholesterol picked up in the periphery can also enter cells through a **scavenger receptor, SR-B1**.
  - This receptor is expressed at high levels in hepatocytes and the steroidogenic tissues, including ovaries, testes, and areas of the adrenal glands.
  - This receptor does not mediate endocytosis of the HDL, but rather transfer of cholesterol into the cell by a mechanism not yet clearly defined.

Atherosclerosis

High-Yield

The metabolism of LDL and HDL intersects in the production and control of fatty streaks and potential plaques in blood vessels. The figure below illustrates one model of atherosclerosis involving HDL and LDL at the site of endothelial cell injury. Damage to the endothelium may be related to many factors, including normal turbulence of the blood, elevated LDL, especially modified or oxidized LDL, free radicals from cigarette smoking, homocystinemia (Chapter 17), diabetes (glycation of LDL), and hypertension. The atherosclerotic lesion represents an inflammatory response sharing several characteristics with granuloma formation, and not simple deposition of cholesterol in the blood vessel.

- Endothelial dysfunction increases adhesiveness and permeability of the endothelium for platelets and leukocytes. Infiltrations involve monocytes and T cells. Damaged endothelium has procoagulant rather than anticoagulant properties. In some cases, the endothelial lining may become partially denuded.
- Local inflammation recruits monocytes and macrophages with subsequent production of reactive oxygen species. LDL can become oxidized and then taken up, along with other inflammatory debris, by macrophages, which can become laden with cholesterol (foam cells). Initially the subendothelial accumulation of cholesterol-laden macrophages produces fatty streaks.
- As the fatty streak enlarges over time, necrotic tissue and free lipid accumulates, surrounded by epithelioid cells and eventually smooth muscle cells, an advanced plaque with a fibrous cap. The plaque eventually begins to occlude the blood vessel, causing ischemia and infarction in the heart, brain, or extremities.

- Eventually the fibrous cap may thin, and the plaque becomes unstable, leading to rupture and thrombosis.
- HDL may be protective by picking up accumulating cholesterol before the advanced lesion forms. ApoA-1 activates LCAT, which in turn adds a fatty acid to cholesterol to produce a cholesterol ester that dissolves in the core of the HDL.
- The HDL may subsequently be picked up by the liver through the apoE receptor or deliver cholesterol through the scavenger receptor SR-B1 (reverse cholesterol transport from the periphery to the liver). The HDL may also transfer the cholesterol to an IDL, reforming a normal, unoxidized LDL particle.

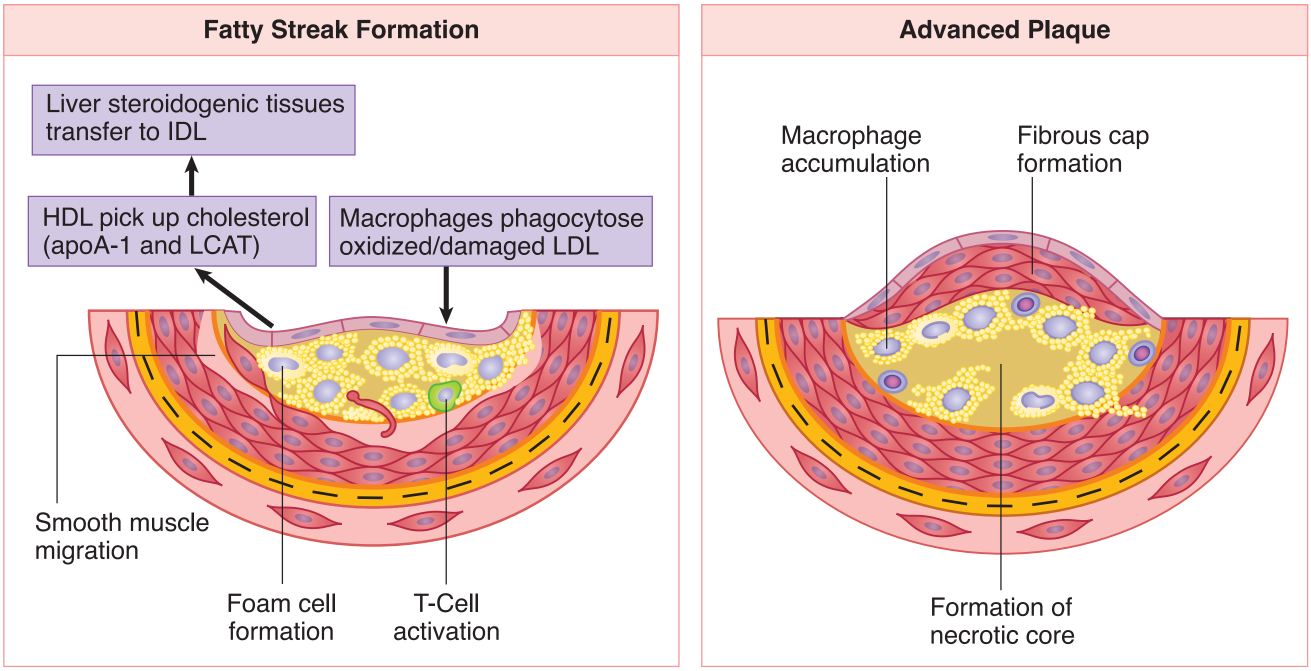


Figure I-15-8. LDL, HDL, and Atherogenesis

Role of Vitamin E

The oxidation of LDL at sites of endothelial damage is thought to be a major stimulus for uptake by macrophages. Some studies have shown a protective role of vitamin E in this process. Vitamin E is a lipid-soluble vitamin that acts as an antioxidant in the lipid phase. In addition to protecting LDL from oxidation, vitamin E may also prevent peroxidation of membrane lipids. Vitamins C and A lack this protective effect despite their antioxidant properties.

Recall Question

Insulin-induced lipoprotein lipase is required for the metabolism of which of the following particles?

A. Chylomicrons

B. IDL

C. LDL

D. Triglycerides

Answer: A

HYPERLIPIDEMIAS

Excess lipid in the blood can result from a primary genetic deficiency (rare) or as a secondary consequence of another disease. There are 2 primary hyperlipidemias: type I hypertriglyceridemia and type IIa hypercholesterolemia.

Table I-15-2. Primary Hyperlipidemias

Type	Deficiency	Lipid Elevated in Blood	Lipoprotein Elevated in Blood	Comments
I	Familial lipoprotein lipase (rare) apoC-II (rare) Autosomal recessive	Triglyceride	Chylomicrons	Red-orange eruptive xanthomas Fatty liver Acute pancreatitis Abdominal pain after fatty meal
IIa	Familial hypercholesterolemia Autosomal dominant (Aa 1/500, AA 1/10^6) LDL-receptor (LDL-R) deficiency	Cholesterol	LDL	High risk of atherosclerosis and coronary artery disease Homozygous condition, usually death $<$20 years Xanthomas of the Achilles tendon Tuberous xanthomas on elbows Xanthelasmas Corneal arcus

Type I Hypertriglyceridemia

High-Yield

Rare genetic absence of lipoprotein lipase results in excess triglyceride in the blood and its deposition in several tissues, including liver, skin, and pancreas. Orange-red eruptive xanthomas over the mucous membranes and skin may be seen. Abdominal pain and acute pancreatitis may occur. Fasting chylomicronemia produces a milky turbidity in the serum or plasma.

Diabetes, alcoholism, and glucose-6-phosphatase deficiency all can produce less severe hypertriglyceridemia with an increase in VLDL and chylomicrons. Factors contributing to the hyperlipidemia are:

- Decreased glucose and triglyceride uptake in adipose tissue
- Overactive hormone-sensitive lipase (Chapter 16)
- Underactive lipoprotein lipase

Hyperlipidemia Secondary to Diabetes

> A 20-year-old man was studying for his final exams and became hungry. He drove to the nearest fast food restaurant and ordered a double cheeseburger, extra large French fries, and a large soda. About an hour later, he developed serious abdominal distress, became nauseated, and was close to fainting. Upon his arrival at the emergency room, tests showed that he was hyperglycemic, as well as hypertriglyceridemic. His cholesterol levels were only slightly elevated. Additional information revealed that he was diabetic, and he recovered quickly after the administration of insulin.

The most common type of hyperlipidemia is type V, in which patients have elevated serum triglycerides in VLDL and chylomicrons in response to a meal containing carbohydrates and fat, respectively. One of the important regulatory functions of insulin in adipose tissue is promoting lipoprotein lipase activity by increasing transcription of its gene. Therefore, the consequence in diabetes is abnormally low levels of lipoprotein lipase and the inability to adequately degrade the serum triglycerides in lipoproteins to facilitate the uptake of fatty acids into adipocytes.

Type IIa Hypercholesterolemia (LDL Receptor Deficiency)

High-Yield

This is an autosomal dominant genetic disease affecting 1/500 (heterozygous) individuals in the United States. It is characterized by elevated LDL cholesterol and increased risk for atherosclerosis and coronary artery disease. Cholesterol deposits may be seen as:

- Xanthomas of the Achilles tendon
- Subcutaneous tuberous xanthomas over the elbows
- Xanthelasma (lipid in the eyelid)
- Corneal arcus

Homozygous individuals ($1/10^6$) often have myocardial infarctions before 20 years of age.

Abetalipoproteinemia (a Hypolipidemia)

High-Yield

Abetalipoproteinemia and hypobetalipoproteinemia are rare conditions that illustrate the importance of lipid absorption and transport. Patients have low to absent serum apoB-100 and apoB-48. Serum triglycerides may be near zero, and cholesterol extremely low.

Because chylomicron levels are very low, fat accumulates in intestinal enterocytes and in hepatocytes. Essential fatty acids and vitamins A and E are not well absorbed. Symptoms in severe cases include:

- Steatorrhea
- Cerebellar ataxia
- Pigmentary degeneration in the retina

- Acanthocytes (thorny appearing erythrocytes)
- Possible loss of night vision

CHOLESTEROL METABOLISM

Cholesterol is required for membrane synthesis, steroid and vitamin D synthesis, and in the liver, bile acid synthesis. Most cells derive their cholesterol from LDL or HDL, but some cholesterol may be synthesized *de novo*. Most *de novo* synthesis occurs in the liver, where cholesterol is synthesized from acetyl-CoA in the cytoplasm. The citrate shuttle carries mitochondrial acetyl-CoA into the cytoplasm, and NADPH is provided by the HMP shunt and malic enzyme.

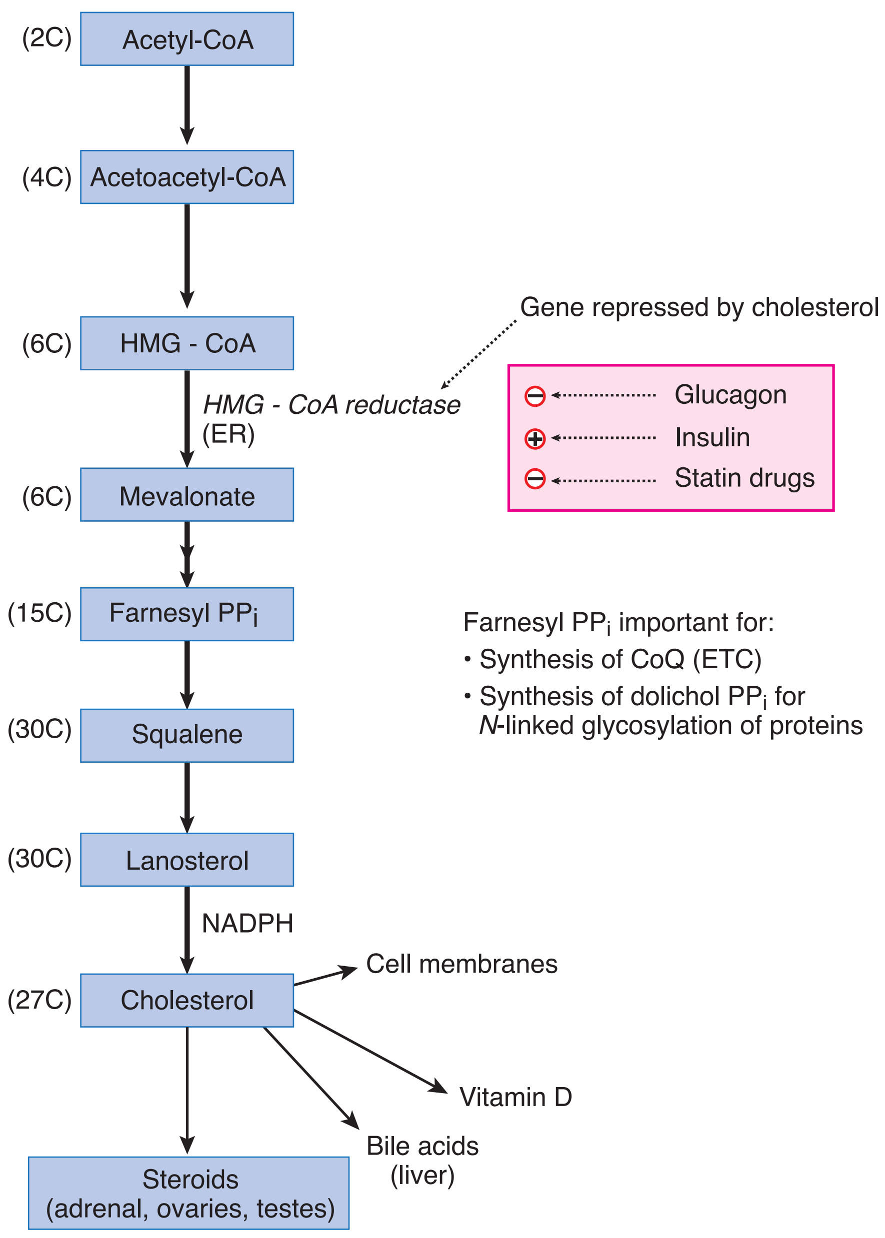


Figure I-15-9. Synthesis of Cholesterol

Bridge to Pharmacology

Treatment of Hypercholesterolemia

Cholestyramine and other drugs that increase elimination of bile salts force the liver to increase their synthesis from cholesterol, thus lowering the internal level of cholesterol in the hepatocytes. Decreased cholesterol within the cell increases LDL receptor expression, allowing the hepatocyte to remove more LDL cholesterol from the blood.

HMG-CoA reductase inhibitors such as atorvastatin and simvastatin inhibit *de novo* cholesterol synthesis in the hepatocyte, which subsequently increases LDL receptor expression.

3-hydroxy-3-methylglutaryl (HMG)-CoA reductase on the smooth endoplasmic reticulum (SER) is the rate-limiting enzyme. Insulin activates the enzyme (dephosphorylation), and glucagon inhibits it. Mevalonate is the product, and the statin drugs competitively inhibit the enzyme. Cholesterol represses the expression of the HMG-CoA reductase gene and also increases degradation of the enzyme.

Farnesyl pyrophosphate, an intermediate in the pathway, may also be used for:

- Synthesis of CoQ for the mitochondrial electron transport chain
- Synthesis of dolichol pyrophosphate, a required cofactor in *N*-linked glycosylation of proteins in the endoplasmic reticulum
- Prenylation of proteins (a posttranslational modification) that need to be held in the cell membrane by a lipid tail. An example is the $p21^{ras}$ G protein in the insulin and growth factor pathways.

Hypercholesterolemia

> A 55-year-old man went to see his physician for his annual checkup. Over the past 5 years, his bloodwork consistently showed that his total cholesterol was slightly high (205–220 mg/dL), HDL 45–50 mg/dL, and LDL 145 mg/dL. Serum C-reactive protein (CRP) value was always within normal range. Despite considerable efforts to lower his total cholesterol and LDL (exercise, 1 glass of wine per day, and a carefully controlled diet), his blood cholesterol values did not significantly change. No other problems were evident. He recently heard that the ideal blood level of LDL should be <100 mg/dL. His physician decided to prescribe a statin drug. Within several weeks of taking the statin, the patient experienced more than usual muscle soreness, pain, and weakness when he exercised. He also noticed that his urine was red-brown.

For a large majority of people, statin drugs work efficiently and without side effects. Maximum lowering of blood cholesterol takes about 4–6 weeks. Because the liver is the site of most cholesterol synthesis, LFTs should be measured 2–3 months after starting the statin regimen. One consequence of statins inhibiting HMG-CoA reductase is the lessening of the downstream synthesis of CoQ, which is needed for the electron transport chain. Without properly functioning mitochondria, muscle would have a decreased ability to generate ATP required for muscle contraction. The red-brown urine is caused by the spillage of myoglobin from damaged muscle cells. CRP is a liver protein that is secreted in inflammation. There is a direct correlation between elevated CRP and atherosclerosis.

Review Questions

Select the ONE best answer.

1. What is the most positive activator of the process shown below?

8 Acetyl-CoA + n ATP + 14 NADPH → palmitate + 8 CoASH + nADP + nPi + 14 NADP

   A. Acetyl-CoA
   B. Citrate
   C. Malonyl-CoA
   D. Malate
   E. Oxaloacetate

2. When adipose tissue stores triglyceride arriving from the liver or intestine, glycolysis must also occur in the adipocyte. Which of the following products or intermediates of glycolysis is required for fat storage?

   A. Glycerol
   B. Glucose 6-phosphate
   C. Pyruvate
   D. Acetyl-CoA
   E. Dihydroxyacetone phosphate

Items 3 and 4

Abetalipoproteinemia is a genetic disorder characterized by malabsorption of dietary lipid, steatorrhea (fatty stools), accumulation of intestinal triglyceride, and hypolipoproteinemia.

3. A deficiency in the production of which apoprotein would most likely account for this clinical presentation?

   A. ApoB-100
   B. ApoB-48
   C. ApoC-II
   D. ApoA-I
   E. ApoE

4. Patients with abetalipoproteinemia exhibit membrane abnormalities in their erythrocytes with production of acanthocytes (thorny-appearing cells). This unusual red cell morphology would most likely result from malabsorption of

   A. palmitic acid
   B. ascorbic acid
   C. arachidonic acid
   D. folic acid
   E. linoleic acid

5. A patient with a history of recurring attacks of pancreatitis, eruptive xanthomas, and increased plasma triglyceride levels (2,000 mg/dl) associated with chylomicrons, most likely has a deficiency in

   A. lipoprotein lipase
   B. LDL receptors
   C. HMG-CoA reductase
   D. apoB-48
   E. apoB-100 receptor

6. Uncontrolled phagocytosis of oxidized LDL particles is a major stimulus for the development of foam cells and fatty streaks in the vascular subendothelium. This process may be inhibited by increased dietary intake of

   A. vitamin E
   B. vitamin B_6
   C. vitamin D
   D. vitamin B_{12}
   E. vitamin K

Items 7–9

A 42-year-old man presents with a chief complaint of intermittent claudication during exercise. His family history is significant for the presence of cardiovascular disease on his father's side, but not on his mother's side. Physical exam reveals xanthelasmas and bilateral tendon xanthomas. A plasma lipid profile reveals cholesterol level 340 mg/dL, with a high LDL/HDL ratio. He is given instructions for dietary modifications and a prescription for simvastatin.

7. The clinical findings noted in this patient are most likely caused by deficient production of

   A. lethicin cholesterol acyltransferase
   B. apoB-100 receptors
   C. fatty acyl-CoA synthetase
   D. VLDL from LDL
   E. cholesterol ester transfer protein

8. The anticholesterolemic action of simvastatin is based on its effectiveness as a competitive inhibitor of the rate-limiting enzyme in cholesterol biosynthesis. The reaction product normally produced by this enzyme is

   A. squalene
   B. methylmalonate
   C. lanosterol
   D. mevalonate
   E. acetoacetate

9. From a Lineweaver-Burk plot, the K_m and V_{max} of this rate-limiting enzyme were calculated to be 4×10^{-3} M and 8×10^2 mmol/h, respectively. If the given experiment is repeated in the presence of simvastatin, which of the following values would be obtained?

	K_m (M)	V_{max} (mmol/h)
A.	4×10^{-3}	3×10^2
B.	2×10^{-3}	1×10^2
C.	4×10^{-3}	9×10^2
D.	8×10^{-3}	8×10^2
E.	8×10^{-3}	9×10^2

10. A 20-year-old man is taken to the university clinic to determine the cause of recurring hyperlipidemia, proteinuria, and anemia. Fasting blood tests reveal slightly elevated concentrations of unesterified cholesterol and phosphatidylcholine. The patient is given a 100 gram chocolate bar and blood lipid levels are monitored hourly. Results reveal significantly increased levels of unesterified cholesterol and phosphatidylcholine for extended periods. Deficiency of which of the following proteins is most likely to be associated with these observations?

A. Acyl-CoA:cholesterol acyltransferase (ACAT)
B. Apoprotein A-1
C. Apoprotein B48
D. Apoprotein B100
E. Lipoprotein lipase

Answers

1. **Answer: B.** Citrate is a potent activator of acetyl-CoA carboxylase for fatty acid synthesis.

2. **Answer: E.** To reform triglycerides from the incoming fatty acids, glycerol 3-P must be available. The adipose can produce this only from DHAP in glycolysis.

3. **Answer: B.** ApoB-48 is required for intestinal absorption of dietary fat in the form of chylomicrons. ApoB-100 formation is also impaired in these patients, but this would not explain the clinical symptoms described.

4. **Answer: E.** The genetic defect would result in malabsorption of the 3 fatty acids listed, but only linoleate is strictly essential in the diet. Absorption of water-soluble ascorbate and folate would not be significantly affected.

5. **Answer: A.** These are the clinical features of lipoprotein lipase deficiency (type I lipoproteinemia). LDL receptor defects would result in elevated LDLs. HMG-CoA reductase and ApoB-100 have no direct relationship to chylomicrons. ApoB-48 deficiency would result in decreased production of chylomicrons.

6. **Answer: A.** Only vitamin E is an antioxidant.

7. **Answer: B.** The findings are indicative of heterozygous type lla familial hypercholesterolemia, an autosomal dominant disease. Deficient CETP, LCAT or fatty acid-CoA synthetase would not elevate LDL cholesterol. VLDL are not produced from LDL.

8. **Answer: D.** Must know that mevalonate precedes squalene and lanosterol in the pathway, and that methylmalonate and acetoacetate are not associated with cholesterolgenesis.

9. **Answer: D.** With a competitive inhibitor, there will be an increase in K_m with no change in V_{max}. Choice A would be for a noncompetitive inhibitor (V_{max} decreased, K_m unaltered).

10. **Answer: B.** Apo A-1 is present on the surface of HDL and functions to activate circulating LCAT (lecithin:cholesterol acyltransferase) to esterify cholesterol, using lecithin (phosphatidylcholine) as the fatty acid donor. Esterification of cholesterol in blood occurs to trap the resultant cholesterol esters in HDL where they can subsequently be transferred to other lipoproteins (IDL) or taken up directly by hepatocytes and steroidogenic tissues.

    Deficiencies of apoprotein B100 and apoprotein B48 (**choices D and C**) result in abetalipoproteinemia characterized by decreased blood triglycerides and cholesterol.

    ACAT (**choice A**) is an intracellular enzyme and esterifies cholesterol inside cells, but not in blood.

    Deficiency of LPL (**choice E**) would result in type 1 hyperlipidemia and is characterized by elevated serum triglycerides.

Lipid Mobilization and Catabolism 16

Learning Objectives

- ❏ Understand the concept of lipid mobilization
- ❏ Interpret scenarios about fatty acid oxidation
- ❏ Demonstrate understanding of ketone body metabolism
- ❏ Know the sphingolipidoses

LIPID MOBILIZATION

In the postabsorptive state, fatty acids can be released from adipose tissue to be used for energy. Although human adipose tissue does not respond directly to glucagon, the fall in insulin activates a hormone-sensitive triacylglycerol lipase (HSL) that hydrolyzes triglycerides, yielding fatty acids and glycerol. Epinephrine and cortisol also activate HSL.

Glycerol may be picked up by liver and converted to dihydroxyacetone phosphate (DHAP) for gluconeogenesis, and the fatty acids are distributed to tissues that can use them. Free fatty acids are transported through the blood in association with serum albumin.

Bridge to Pharmacology

Niacin is a commonly used antihyperlipidemic drug. In large doses (gram-level quantities that are well above the RDA), it works by inhibiting HSL in adipose tissue. With fewer fatty acids entering the liver, VLDL will not be assembled in normal amounts. Both VLDL (carrying triglycerides and cholesterol) and its product, LDL, will be lower in serum.

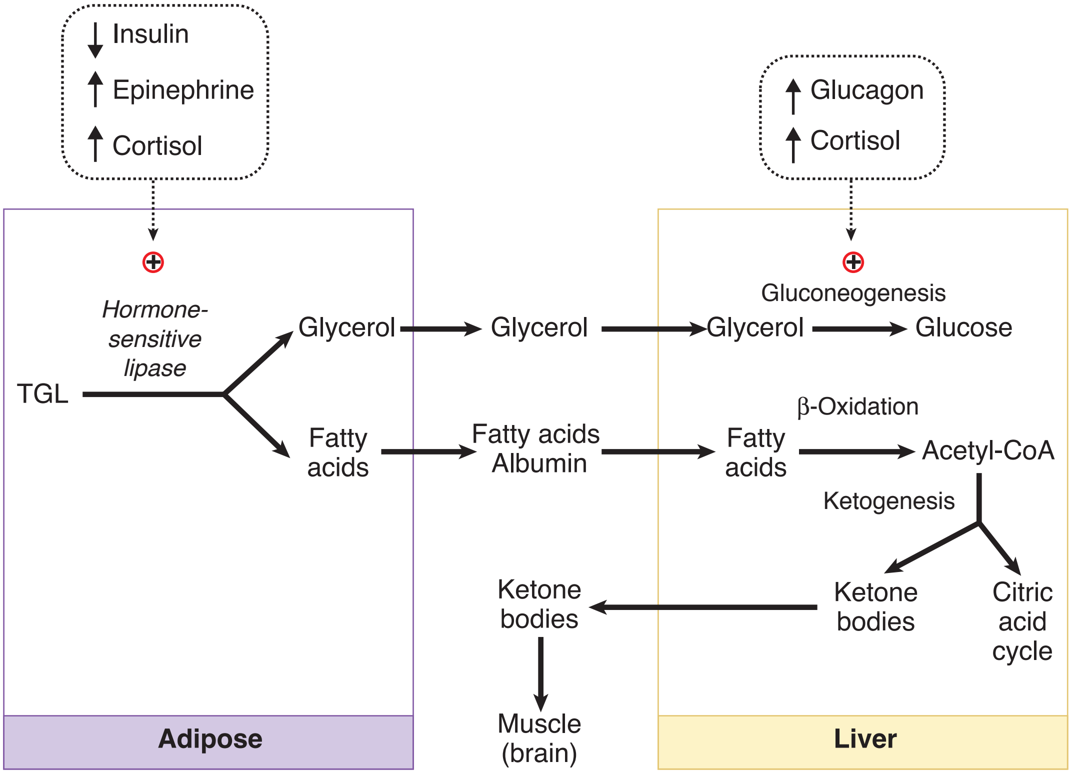


Figure I-16-1. Lipolysis of Triglyceride in Response to Hypoglycemia and Stress

FATTY ACID OXIDATION

Fatty acids are oxidized in several tissues, including liver, muscle, and adipose tissue, by the pathway of β-oxidation. Neither erythrocytes nor brain can use fatty acids and so continue to rely on glucose during normal periods of fasting. Erythrocytes lack mitochondria, and fatty acids do not cross the blood–brain barrier efficiently.

Short-chain fatty acids (2–4 carbons) and medium-chain fatty acids (6–12 carbons) diffuse freely into mitochondria to be oxidized. Long-chain fatty acids (14–20 carbons) are transported into the mitochondrion by a carnitine shuttle to be oxidized. Very long-chain fatty acids (>20 carbons) enter peroxisomes via an unknown mechanism for oxidation.

Fatty Acid Entry into Mitochondria

High-Yield

Long-chain fatty acids must be activated and transported into the mitochondria. Fatty acyl-CoA synthetase, on the outer mitochondrial membrane, activates the fatty acids by attaching CoA. The fatty acyl portion is then transferred onto carnitine by carnitine acyltransferase-1 for transport into the mitochondria. The sequence of events includes the following steps:

- Fatty acyl synthetase activates the fatty acid (outer mitochondrial membrane).
- Carnitine acyltransferase-1 transfers the fatty acyl group to carnitine (outer mitochondrial membrane).
- Fatty acylcarnitine is shuttled across the inner membrane.
- Carnitine acyltransferase-2 transfers the fatty acyl group back to a CoA (mitochondrial matrix).

Carnitine acyltransferase-1 is inhibited by malonyl-CoA from fatty acid synthesis and thereby prevents newly synthesized fatty acids from entering the mitochondria. Insulin indirectly inhibits β-oxidation by activating acetyl-CoA carboxylase (fatty acid synthesis) and increasing the malonyl-CoA concentration in the cytoplasm. Glucagon reverses this process.

β-Oxidation in Mitochondria

High-Yield

β-oxidation reverses the process of fatty acid synthesis by oxidizing (rather than reducing) and releasing (rather than linking) units of acetyl-CoA. The pathway is a repetition of 4 steps. Each 4-step cycle releases one acetyl-CoA and reduces NAD and FAD (producing NADH and $FADH_2$).

The $FADH_2$ and NADH are oxidized in the electron transport chain, providing ATP. In muscle and adipose tissue, the acetyl-CoA enters the citric acid cycle. In liver, the ATP may be used for gluconeogenesis, and the acetyl-CoA (which cannot be converted to glucose) stimulates gluconeogenesis by activating pyruvate carboxylase.

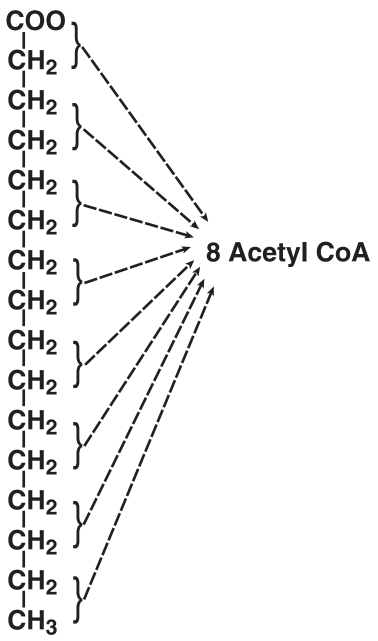
In a fasting state, the liver produces more acetyl-CoA from β-oxidation than is used in the citric acid cycle. Much of the acetyl-CoA is used to synthesize ketone bodies (essentially 2 acetyl-CoA groups linked together) that are released into the blood for other tissues.

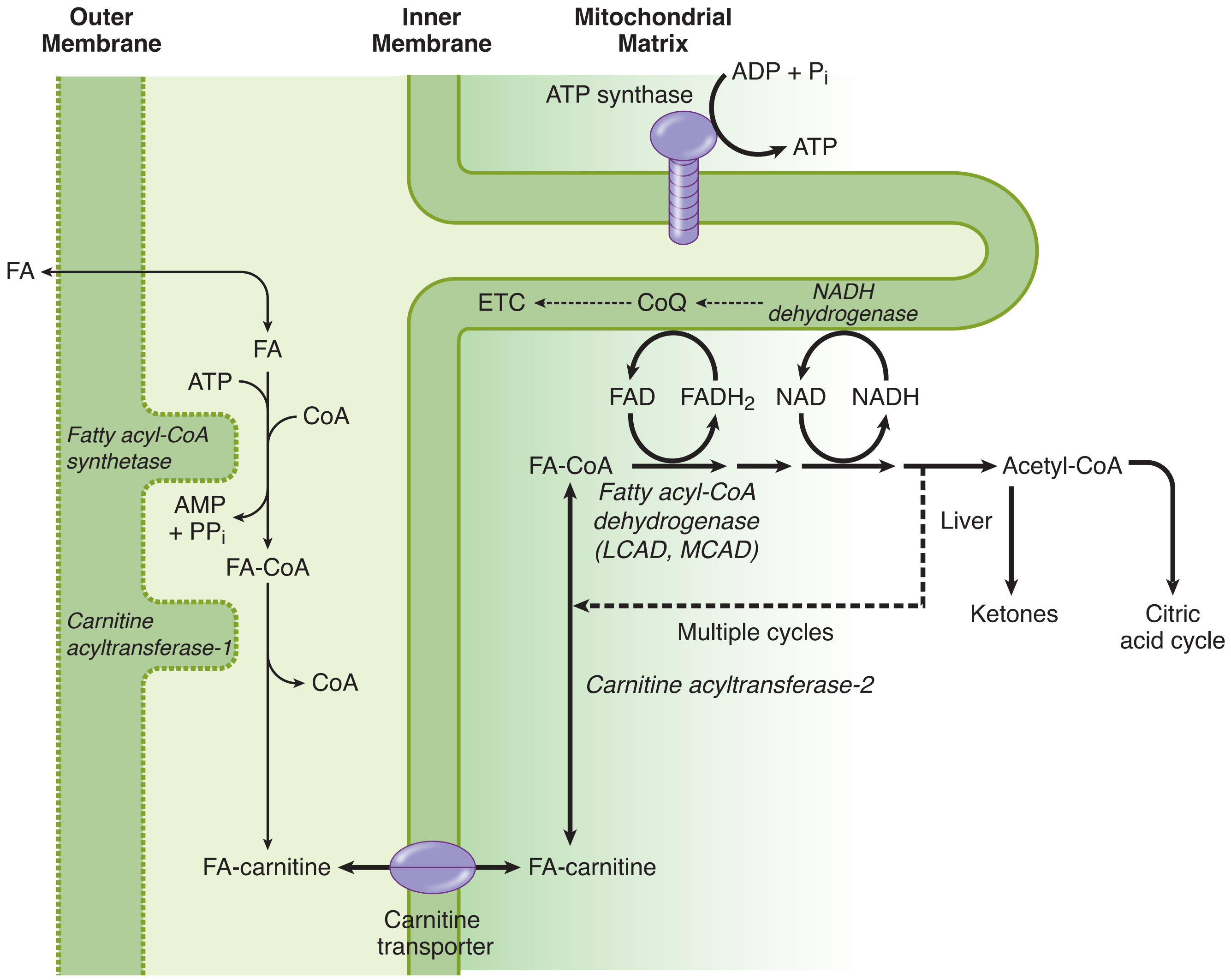
Note

Carnitine acyltransferase-1 and -2 (CAT-1 and CAT-2) are also referred to as carnitine palmitoyl transferase-1 and -2 (CPT-1 and CPT-2). The carnitine transport system is most important for allowing long-chain fatty acids to enter into the mitochondria.

Note

β-oxidation of palmitate (16:0) yields 8 acetyl-CoA.





Myopathic CAT/CPT Deficiency

- Muscle aches, weakness
- Myoglobinuria
- Provoked by prolonged exercise, especially if fasting
- Biopsy: elevated muscle triglyceride
- Most common form: AR, late-onset

MCAD Deficiency

- Fasting hypoglycemia
- No ketone bodies (hypoketosis)
- C8–C10 acyl carnitines in blood
- Vomiting
- Coma, death
- AR with variable expression
- Dicarboxylic aciduria

Figure I-16-2. Fatty Acid Activation, Transport, and β-Oxidation

Genetic deficiencies of fatty acid oxidation

Two of the most common genetic deficiencies affecting fatty acid oxidation are **medium chain acyl-CoA dehydrogenase (MCAD) deficiency**, primary etiology hepatic, and **myopathic carnitine acyltransferase (CAT/CPT) deficiency**, primary etiology myopathic.

- **MCAD deficiency**: Non-ketotic hypoglycemia should be strongly associated with a block in hepatic β-oxidation. During fasting, hypoglycemia can become profound due to lack of ATP to support gluconeogenesis. Decreased acetyl-CoA lowers pyruvate carboxylase activity and also limits ketogenesis. Hallmarks of this disease include:
  - Profound fasting hypoglycemia
  - Low to absent ketones
  - Lethargy, coma, death if untreated
  - C8–C10 acyl carnitines in blood
  - In an infant, episode may be provoked by overnight fast; in an older child, often provoked by illness (flu) that causes loss of appetite and vomiting
  - Primary treatment: IV glucose
  - Prevention: frequent feeding, high-carbohydrate, low-fat diet

  Most patients lead reasonable lives if they take frequent carbohydrate meals to avoid periods of hypoglycemia. Complications arise when fatty meals are ingested and the MCAD enzyme is required to catabolize them. Also, in times of metabolic stress induced by severe fasting, exercise, or infection (conditions with high demands on fatty acid oxidation), symptoms of MCAD deficiency are manifested.
- **CAT-2/CPT-2 (myopathic form adolescent or adult onset)**: Although all tissues with mitochondria contain carnitine acyltransferase, the most common form of this genetic deficiency is myopathic and due to a defect in the muscle-specific CAT/CPT gene. It is an autosomal recessive condition with late onset. Hallmarks of this disease include:
  - Muscle aches; mild to severe weakness
  - Rhabdomyolysis, myoglobinuria, "red urine"
  - Episode provoked by prolonged exercise especially after fasting, cold, or associated stress
  - Symptoms may be exacerbated by high-fat, low-carbohydrate diet
  - Muscle biopsy shows elevated muscle triglyceride detected as lipid droplets in cytoplasm
  - Primary treatment: cease muscle activity and give glucose

  A somewhat similar syndrome to CAT-2/CPT-2 can be produced by muscle carnitine deficiency secondary to a defect in the transport system for carnitine in muscle.

Bridge to Pathology

It is now believed that some cases of sudden infant death syndrome (SIDS) are due to MCAD deficiency. The incidence of MCAD deficiency is one of the highest of the inborn errors of metabolism (estimated at 1/10,000).

Clinical Correlate

Ackee, a fruit grown in Jamaica and West Africa, contains hypoglycin, a toxin that acts as an inhibitor of fatty acyl-CoA dehydrogenase. Jamaican vomiting sickness and severe hypoglycemia can result if it is ingested. Symptoms include sudden onset of vomiting 2–6 hours after ingesting an ackee-containing meal. After a period of prostration that may last as long as 18 hours, more vomiting may occur, followed by convulsions, coma, and even death.

MCAD Deficiency

A 6-year-old suffered gastroenteritis for 3 days that culminated in a brief generalized seizure, which left him semicomatose. Admitting blood glucose was 45 mg/dL (0.25 mM), and urine was negative for glucose and ketones. Intravenous glucose improved his condition within 10 minutes. Subsequent bloodwork revealed elevated C8–C10 acyl carnitines. Following a diagnosis of an enzyme deficiency, the boy's parents were cautioned to make sure that he eats meals frequently.

The presence of severe hypoglycemia and absence of ketosis (hypoketosis) is strongly suggestive of a block in β-oxidation. The episode in this case was precipitated by the 3-day gastroenteritis (vomiting/fasting). The elevated C8–C10 acyl carnitines accumulate due to the defective MCAD and they leak into serum. These compounds are detected using sophisticated tandem mass spectrometry.

Propionic Acid Pathway

High-Yield

Fatty acids with an odd number of carbon atoms are oxidized by β-oxidation identically to even-carbon fatty acids. The difference results only from the final cycle, in which even-carbon fatty acids yield 2 acetyl-CoA (from the 4-carbon fragment remaining) but odd-carbon fatty acids yield one acetyl-CoA and one propionyl-CoA (from the 5-carbon fragment remaining).

Propionyl-CoA is converted to succinyl-CoA, a citric acid cycle intermediate, in the 2-step propionic acid pathway. Because this extra succinyl-CoA can form malate and enter the cytoplasm and gluconeogenesis, odd-carbon fatty acids represent an exception to the rule that fatty acids cannot be converted to glucose in humans. The propionic acid pathway includes 2 important enzymes, both in the mitochondria:

- Propionyl-CoA carboxylase requires biotin
- Methylmalonyl-CoA mutase requires vitamin B12, cobalamin

Vitamin B12 deficiency can cause a megaloblastic anemia of the same type seen in folate deficiency (chapter 17). In a patient with megaloblastic anemia, it is important to determine the underlying cause because B12 deficiency, if not corrected, produces a peripheral neuropathy owing to aberrant fatty acid incorporation into the myelin sheets associated with inadequate methylmalonyl-CoA mutase activity. Excretion of methylmalonic acid indicates a vitamin B12 deficiency rather than folate.

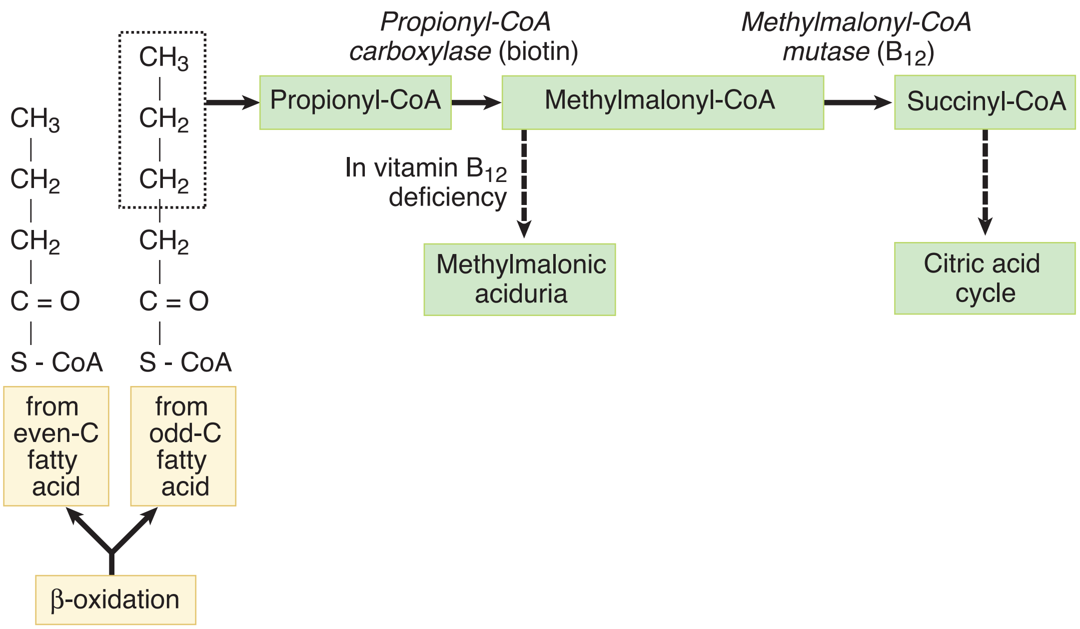


Figure I-16-3. Propionic Acid Pathway

KETONE BODY METABOLISM

In the fasting state, the liver converts excess acetyl-CoA from β-oxidation of fatty acids into ketone bodies, acetoacetate and 3-hydroxybutyrate (β-hydroxybutyrate), which are used by extrahepatic tissues. Cardiac and skeletal muscles and renal cortex metabolize acetoacetate and 3-hydroxybutyrate to acetyl-CoA.

Normally during a fast, muscle metabolizes ketones as rapidly as the liver releases them, preventing their accumulation in blood.

After a week of fasting, ketones reach a concentration in blood high enough for the brain to begin metabolizing them. If ketones increase sufficiently in the blood, they can lead to ketoacidosis.

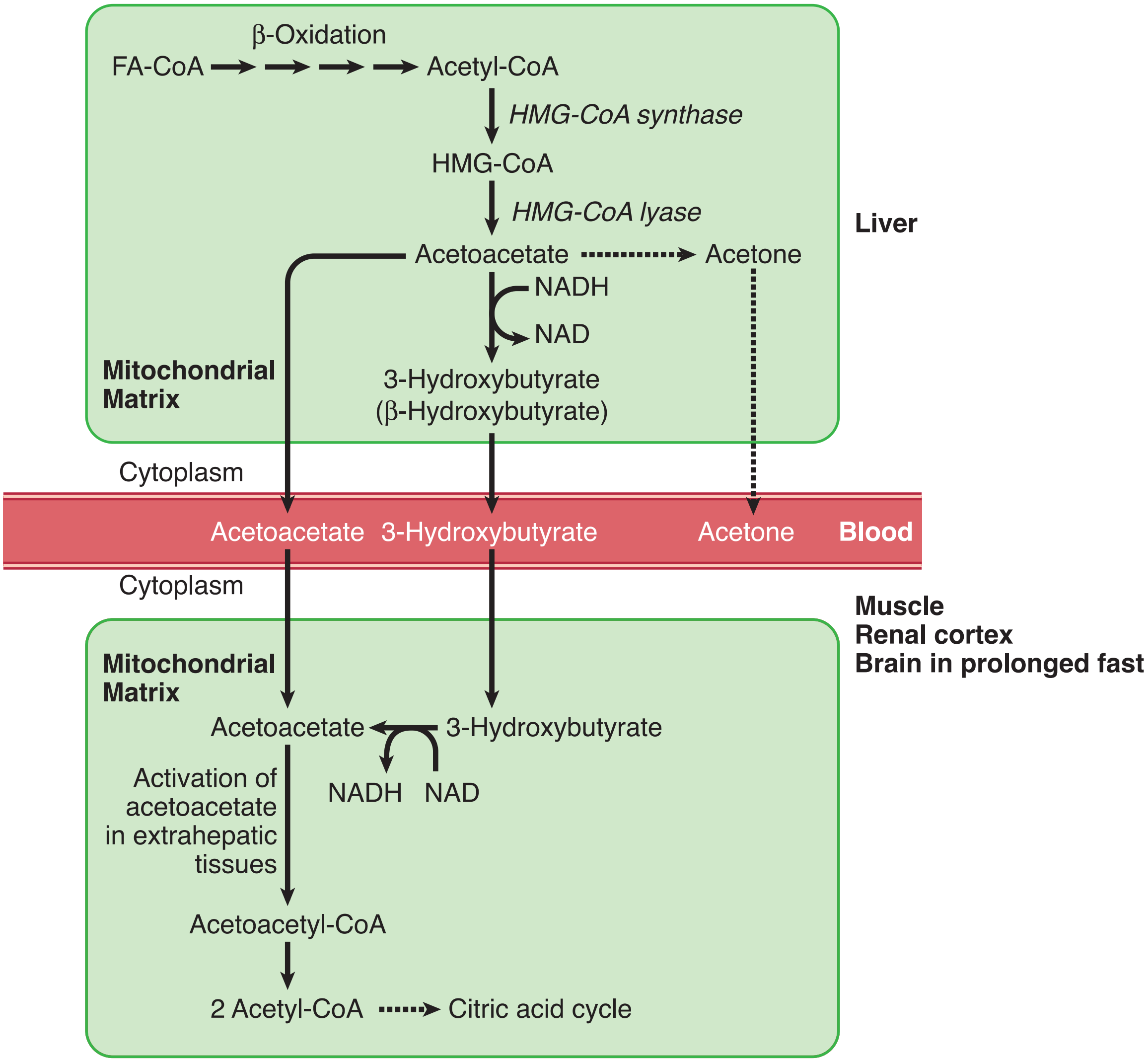


Figure I-16-4. Ketogenesis (Liver) and Ketogenolysis (Extrahepatic)

Clinical Correlate

In untreated type 1 diabetes mellitus, there is no insulin. Although glucose may be high, no insulin is released, HSL is active, β-oxidation is not inhibited, and ketone bodies are generated.

Ketogenesis

High-Yield

Ketogenesis occurs in mitochondria of hepatocytes when excess acetyl-CoA accumulates in the fasting state. HMG-CoA synthase forms HMG-CoA, and HMG-CoA lyase breaks HMG-CoA into acetoacetate, which can subsequently be reduced to 3-hydroxybutyrate. Acetone is a minor side product formed nonenzymatically but is not used as a fuel in tissues. It does, however, impart a strong odor (sweet or fruity) to the breath, which is almost diagnostic for ketoacidosis.

Ketogenolysis

High-Yield

Acetoacetate picked up from the blood is activated in the mitochondria by succinyl-CoA acetoacetyl-CoA transferase (common name thiophorase), an enzyme present only in extrahepatic tissues; 3-hydroxybutyrate is first oxidized to acetoacetate. Because the liver lacks this enzyme, it cannot metabolize the ketone bodies.

The figure below shows the **major pathways producing fuel for the brain**. Note the important times at which the brain switches from:

- Glucose derived from liver glycogenolysis to glucose derived from gluconeogenesis (~12 hours)
- Glucose derived from gluconeogenesis to ketones derived from fatty acids (~1 week)

In the brain, when ketones are metabolized to acetyl-CoA, pyruvate dehydrogenase is inhibited. Glycolysis and subsequently glucose uptake in brain decreases. This important switch spares body protein (which otherwise would be catabolized to form glucose by gluconeogenesis in the liver) by allowing the brain to indirectly metabolize fatty acids as ketone bodies.

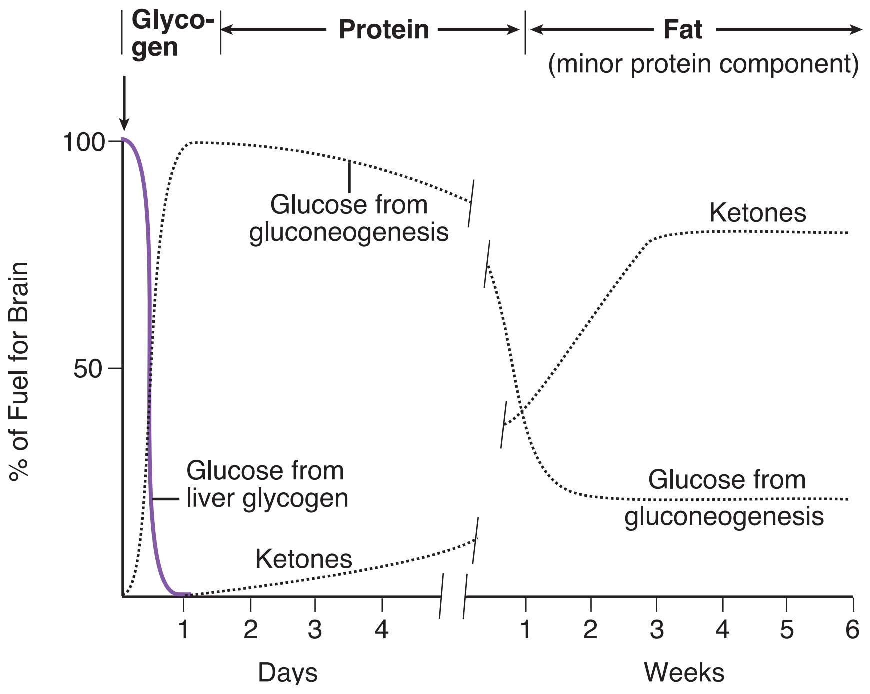


Figure I-16-5. Fuel Use in the Brain During Fasting and Starvation

Ketoacidosis

High-Yield

- In patients with type 1 insulin-dependent diabetes mellitus not adequately treated with insulin, fatty acid release from adipose tissue and ketone synthesis in the liver exceed the ability of other tissues to metabolize them, and a profound, life-threatening ketoacidosis may occur. An infection or trauma (causing an increase in cortisol and epinephrine) may precipitate an episode of ketoacidosis by activating HSL.
- Patients with type 2 non–insulin-dependent diabetes mellitus (NIDDM) are much less likely to show ketoacidosis. The basis for this observation is not completely understood, although type 2 disease has a much slower, insidious onset, and insulin resistance in the periphery is usually not complete. Type 2 diabetics can develop ketoacidosis after an infection or trauma.

Note

In certain populations with **NIDDM**, ketoacidosis is much more common than previously appreciated.

Alcoholics can also develop ketoacidosis. Chronic hypoglycemia, which is often present in chronic alcoholism, favors fat release from adipose. Ketone production increases in the liver, but utilization in muscle may be slower than normal because alcohol is converted to acetate in the liver, diffuses into the blood, and oxidized by muscle as an alternative source of acetyl-CoA.

Associated with ketoacidosis:

- Polyuria, dehydration, and thirst (exacerbated by hyperglycemia and osmotic diuresis)
- CNS depression and coma
- Potential depletion of K^+ (although loss may be masked by a mild hyperkalemia)
- Decreased plasma bicarbonate
- Breath with a sweet or fruity odor, acetone

Clinical Correlate

Insulin facilitates uptake of potassium by cells, so K^+ levels may be normal before DKA is treated with insulin. Once insulin is administered, however, blood potassium levels need to be monitored.

Laboratory measurement of **ketones** should be done regularly. In **normal ketosis** (which accompanies fasting and does not produce an acidosis), acetoacetate and β-hydroxybutyrate are formed in approximately equal quantities.

In **pathologic conditions** such as diabetes and alcoholism, ketoacidosis may develop with life-threatening consequences. In diabetic and alcoholic ketoacidosis, the ratio between acetoacetate and β-hydroxybutyrate shifts, and β-hydroxybutyrate predominates.

- The urinary nitroprusside test detects only acetoacetate and can dramatically underestimate the extent of ketoacidosis and its resolution during treatment.
- β-hydroxybutyrate should be measured.

Home monitors of both blood glucose and β-hydroxybutyrate are available for diabetic patients.

Recall Question

Inhibition of which of the following enzymes is responsible for the peripheral neuropathy involved in vitamin B12 deficiency?

A. Carnitine acyltransferase-1

B. Carnitine acyltransferase-2

C. Medium chain acyl-CoA dehydrogenase

D. Methylmalonyl-CoA mutase

E. Propionyl-CoA carboxylase

Answer: D

SPHINGOLIPIDS

Sphingolipids are important constituents of cell membranes. Although they contain no glycerol, they are similar in structure to the glycerophospholipids in that they have a hydrophilic region and 2 fatty acid–derived hydrophobic tails.

The various classes of sphingolipids differ primarily in the nature of the hydrophilic region. Classes of sphingolipids and their hydrophilic groups include:

- Sphingomyelin: phosphorylcholine
- Cerebrosides: galactose or glucose
- Gangliosides: branched oligosaccharide chains terminating in the 9-carbon sugar, sialic acid (*N*-acetylneuraminic acid, NANA)

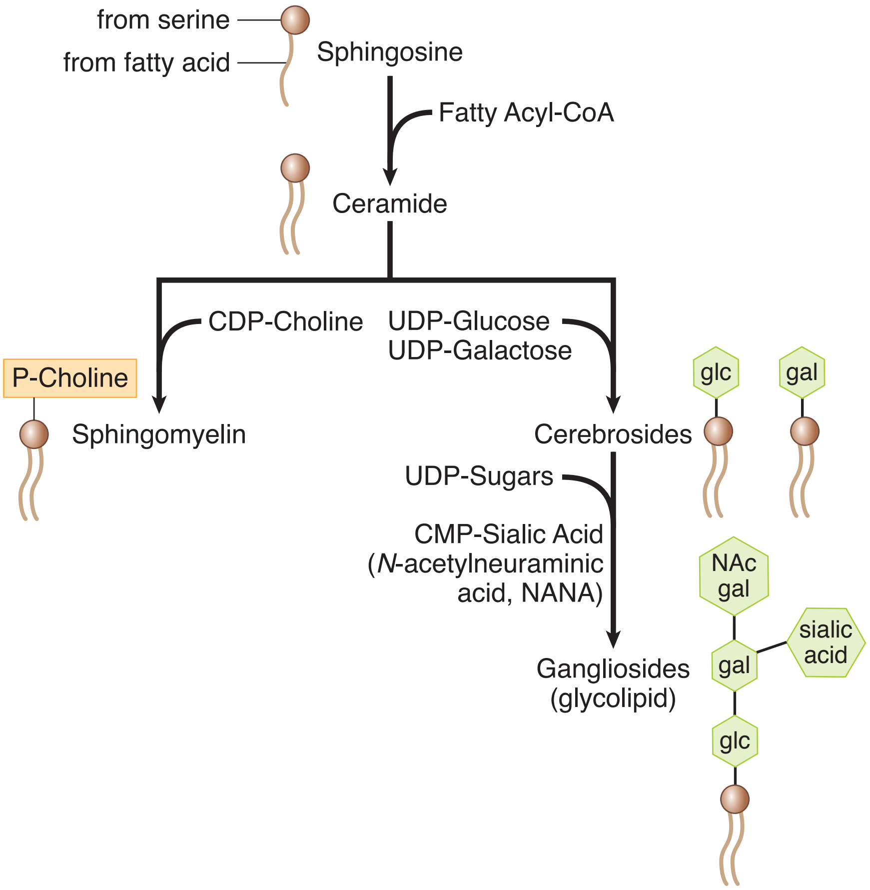


Figure I-16-6. Synthesis of Sphingolipids

Genetic Deficiencies of Enzymes in Sphingolipid Catabolism

High-Yield

Sphingolipids released when membrane is degraded are digested in endosomes after fusion with lysosomes. Lysosomes contain many enzymes, each of which removes specific groups from individual sphingolipids. Genetic deficiencies of many of these enzymes are known, and the diseases share some of the characteristics of I-cell disease discussed in Chapter 4.

Table I-16-1. Genetic Deficiencies of Sphingolipid Catabolism

Disease	Lysosomal Enzyme Missing	Substrate Accumulating in Inclusion Body	Symptoms
Tay-Sachs	Hexosaminidase A	Ganglioside GM_2	• Cherry red spots in macula • Blindness • Psychomotor retardation • Death usually <2 years • Startle reflex
Gaucher	Glucocerebrosidase	Glucocerebroside	• Type 1 (99%): adult hepatosplenomegaly • Erosion of bones, fractures • Pancytopenia or thrombocytopenia • Characteristic macrophages • (crumpled paper inclusions)
Niemann-Pick	Sphingomyelinase	Sphingomyelin	• May see cherry red spot in macula • Hepatosplenomegaly • Microcephaly, severe mental retardation • Foamy macrophages with zebra bodies • Early death

Gaucher Disease

A 9-year-old boy of Ashkenazic Jewish descent was brought to the university hospital because he was extremely lethargic and bruised easily. The attending physician noted massive hepatomegaly and splenomegaly, marked pallor, and hematologic complications. A histologic examination obtained from bone marrow showed a "wrinkled" appearance of the cytoplasm and that the cytoplasm was highly PAS stain positive. White cells were taken to assay for glucocerebrosidase, and the activity of the enzyme was found to be markedly below normal.

Outside the brain, glucocerebroside arises mainly from the breakdown of old red and white blood cells. In the brain, glucocerebroside arises from the turnover of gangliosides during brain development and formation of the myelin sheath. Without the proper degradation of glucocerebroside due to a lack of glucocerebrosidase, it accumulates in cells and tissues responsible for its turnover. This results in "wrinkled-paper" cytoplasm and carbohydrate positive staining. The easy bruising is due to a low blood platelet count, and the lethargy is due to the anemia.

Highly effective enzyme replacement therapy is available for Gaucher patients. Enzyme replacement results in the reduction of hepatosplenomegaly, skeletal abnormalities, and other Gaucher-associated problems. The major drawback of therapy using intravenously administered recombinant glucocerebrosidase is its prohibitive cost (several hundred thousand dollars per year).

Note

Study sphingolipid catabolism for the exam. Be sure to know the diseases and their clinical symptoms, enzyme that's missing, and the substrate that accumulates.

Fabry Disease

In contrast to the other sphingolipidoses (Tay-Sachs, Gaucher, Niemann-Pick) which are all autosomal recessive, Fabry disease is the only one that is X-linked recessive. Fabry is caused by a mutation in the gene that encodes the lysosomal enzyme alpha-galactosidase. Ceramide trihexoside accumulates in the lysosomes. Fabry disease presents during childhood or adolescence:

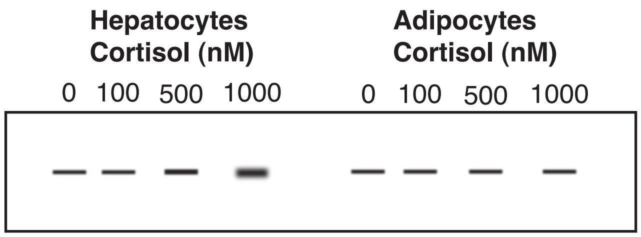
- Burning sensations in the hands which gets worse with exercise and hot weather
- Small, raised reddish-purple blemishes on the skin (angiokeratomas)
- Eye manifestations, especially cloudiness of the cornea
- Impaired arterial circulation and increased risk of heart attack or stroke
- Enlargement of the heart and kidneys
- Often there is survival into adulthood but with increased risk of cardiovascular disease, stroke.
- Renal failure is often the cause of death.

Enzyme replacement therapy is available and, although expensive, slows the progression of the disease.

Review Questions

Select the ONE best answer.

1. As part of a study to quantify contributors of stress to hyperglycemia and ketosis in diabetes, normal hepatocytes and adipocytes in tissue culture were treated with cortisol and analyzed by Northern blotting using a gene-specific probe. The results of one experiment are shown below.



The ^{32}P-probe used in this experiment most likely binds to a mRNA encoding

A. phosphoenolpyruvate carboxykinase

B. lipoprotein lipase

C. glucokinase

D. hormone-sensitive lipase

E. acetyl-CoA carboxylase

2. A child is diagnosed with a congenital deficiency of medium-chain acyl-CoA dehydrogenase activity. Which of the following signs or symptoms would most likely occur upon fasting in this child?

A. Hypolacticacidemia

B. Ketoacidosis

C. Hyperglycemia

D. Dicarboxylic acidosis

E. Hyperchylomicronemia

3. A 3-year-old child complains of muscle pain and weakness while in the playground and is admitted to the hospital for examination. Tests reveal slight hepatomegaly and cardiomegaly. A liver biopsy shows extreme but nonspecific fatty changes, and a muscle biopsy contains large amounts of cytoplasmic vacuoles containing neutral lipid. A one-day fast is performed and shows a drop in blood glucose levels without a corresponding production of ketone bodies. The pH of the blood is normal. Which of the following diagnoses might account for this child's problems?

A. Bilirubin diglucuronide transporter deficiency

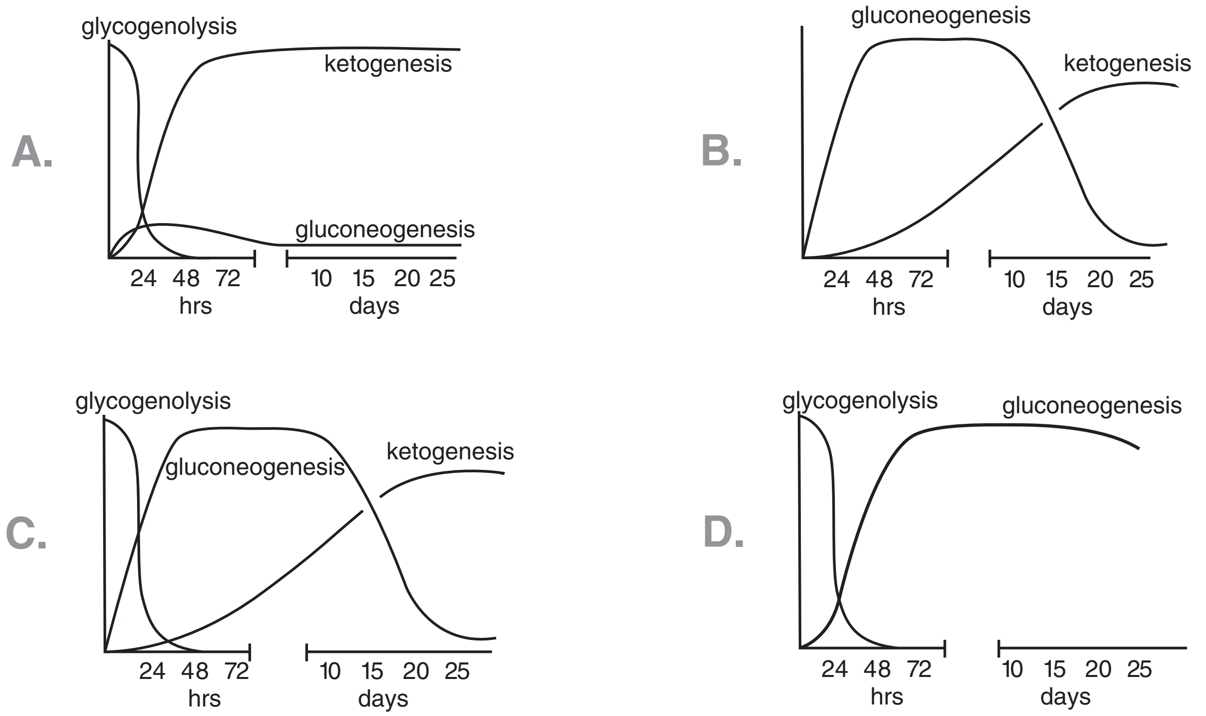
B. Glucose 6-phosphatase deficiency

C. Mitochondrial 3-hydroxy 3-methylglutaryl-CoA synthase deficiency

D. Systemic carnitine deficiency

E. Vitamin D deficiency

Items 4–6



In the options above, each graph depicts the primary source of fuel used by the brain during fasting/starvation. For each condition listed below, select the most closely matched graph.

4. Normal individual

5. Liver phosphorylase deficiency

6. Hepatic fructose-1,6-bisphosphatase deficiency

Items 7–9

A 54-year-old man with type 1 (IDDM) diabetes is referred to an ophthalmologist for evaluation of developing cataracts. Pre-appointment blood work was requested and the results are shown below:

Fasting blood glucose	180 mg/dL
Hemoglobin A	15 gm/dL
Hemoglobin A_{1c}	10% of total Hb
Urine ketones	Positive
Urine glucose	Positive

7. Which of the following enzymes is most strongly associated with cataract formation in this patient?

   A. Galactokinase
   B. Aldose reductase
   C. Glucokinase
   D. Galactose 1-P uridyl transferase
   E. Aldolase B

8. Which of the following best indicates that the blood glucose in this patient has been elevated over a period of weeks?

   A. Presence of ketone bodies
   B. Hyperglycemia
   C. Lipemia
   D. Elevated HbA_{1c}
   E. Lipoprotein lipase

9. Which of the following enzymes would be more active in this patient than in a normal control subject?

   A. Hormone-sensitive lipase
   B. Glucokinase
   C. Fatty acid synthase
   D. Glycogen synthase
   E. Lipoprotein lipase

10. A 40-year-old woman with a history of bleeding and pancytopenia now presents with leg pain. She describes a deep, dull pain of increasing severity that required pain medication. Computed tomography examination reveals erosion and thinning of the femoral head. A bone marrow biopsy is performed to confirm a diagnosis of Gaucher disease. What material would be found abnormally accumulating in the lysosomes of her cells?

    A. Mucopolysaccharide
    B. Ganglioside
    C. Ceramide
    D. Cerebroside
    E. Sulfatide

11. An underweight 4-year-old boy presents semicomatose in the emergency room at 10 A.M. Plasma glucose, urea, and glutamine are abnormally low; acetoacetate is elevated; and lactate is normal. He is admitted to the ICU, where an increase in blood glucose was achieved by controlled infusion of glucagon or alanine. Which metabolic pathway is most likely deficient in this child?

    A. Hepatic gluconeogenesis
    B. Skeletal muscle glycogenolysis
    C. Adipose tissue lipolysis
    D. Skeletal muscle proteolysis
    E. Hepatic glycogenolysis

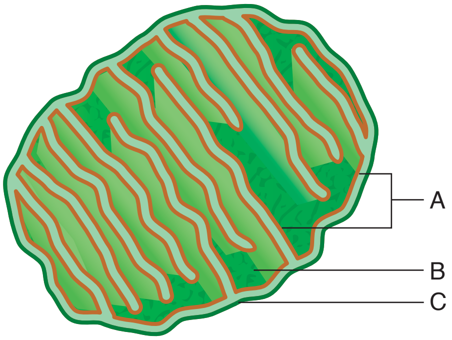
12. After suffering injuries in a motor vehicle accident, a 7-year-old boy undergoes open reduction surgery to repair a compound fractured femur. Post-surgically, the boy undergoes severe hemorrhage and requires transfusion of 8 units of blood. Coagulation studies demonstrate the PT time to be normal, but the PTT time is prolonged. Mixing the boy's plasma with normal plasma returns the PTT time to normal. The mode of inheritance of this boy's disease is most similar to which of the following inherited enzyme deficiencies?

    A. Adenosine deaminase deficiency
    B. α-Galactosidase A deficiency
    C. Glucocerebrosidase deficiency
    D. Hexosaminidase A deficiency
    E. Dystrophia myotonica protein kinase deficiency

13. A 15-month-old female infant is brought to the emergency room by her parents. The infant's mother did not receive routine pre-natal care, and limited information is available regarding the infant's pediatric care. The mother does reveal that the infant "doesn't seem like her other children" and has always been very "fussy."' Physical examination reveals a distressed infant who does not verbalize. Her abdomen is tender and enlargement of both spleen and liver are present. Opthalmoscopic examination fails to reveal cherry-red spots. After a brief hospital course, the infant dies and autopsy is performed. Neural tissue shows parallel striations of electron-dense material within lysosomes. A defect in which of the following was most likely present in this infant?

    A. Golgi-associated phosphate transfer to mannose
    B. Degradation of ganglioside GM2
    C. Degradation of glucocerebrosides
    D. Degradation of sphingomyelin
    E. Synthesis of gangliosides

Items 14-17



For each item listed below, select the appropriate location from the drawing shown above.

14. Carnitine shuttle

15. F_0F_1 ATP synthase

16. HMG-CoA lyase

17. Carnitine palmitoyltransferase-1

Answers

1. **Answer: A.** Cortisol stimulates transcription of the PEP carboxykinase gene in the liver but not in adipose tissue.

2. **Answer: D.** Fasted MCAD patients typically present with nonketotic hypoglycemia, lactic acidosis, and plasma dicarboxylates.

3. **Answer: D.** Upon entry into the playground to have fun, the child secretes epinephrine, which results in adipose tissue triglyceride (TG) breakdown and entry of fatty acids (FA) into muscle and liver for mitochrondrial β-oxidation. A defect in the carnitine shuttle system in this patient would result in accumulation of TG (re-synthesis from FA) in liver and muscle cytoplasm.

   Deficiency of the bilirubin diglucuronide transporter (**choice A**) would result in a liver problem but not the muscle problem that this child has, since the liver processes bilirubin. One would also expect an increase in conjugated bilirubin and clay-colored stools if bilirubin diglucuronide was not entering the bile caniculi.

   Glucose 6-phosphatase deficiency (**choice B**) would account for the hepatomegaly and fatty liver, but not for the muscle weakness. These individuals are also prone to lactic acidosis, which would lower the blood pH.

   Mitochondrial 3-hydroxy 3-methylglutaryl-CoA (HMG-CoA) synthase deficiency (**choice C**) would present somewhat similarly to a β-oxidation deficiency. Prolonged fasting hypoglycemia would provoke release of fatty acids into the blood and their use by tissues such as the muscle and liver. The deficiency of the mitochondrial HMG-Co synthase prevents ketone formation. There would be no muscle weakness because the muscle can use fatty acids. The brain is unable to do so, and the presence of hypoketotic hypoglycemia would deprive the brain of sufficient energy. Coma and death may result.

   Vitamin D deficiency (**choice E**) would not have an effect on organ enlargement and blood glucose levels.

4. **Answer: C.** Glycogen depleted around 18 hours, gluconeogenesis from protein begins to drop gradually, and by 2 weeks, ketones have become the more important fuel for the brain.

5. **Answer: B.** Glycogen would not be mobilized from the liver.

6. **Answer: A.** Gluconeogenesis from proteins would be severely restricted without this enzyme.

7. **Answer: B.** Aldose reductase is rich in lens and nerve tissue (among others) and converts glucose to sorbitol, which causes the osmotic damage. In galactosemia, this same enzyme converts galactose to galactitol, also creating cataracts.

8. **Answer: D.** HbA_{1c} is glycosylated HbA and is produced slowly whenever the glucose in blood is elevated. It persists until the RBC is destroyed and the Hb degraded and so is useful as a long-term indicator of glucose level.

9. **Answer: A.** Because the diabetes is not being well controlled, assume the response to insulin is low and the man would have overstimulated glucagon pathways.

10. **Answer: D.** Glucocerebrosides would accumulate in the cells because the missing enzyme is glucocerebrosidase.

11. **Answer: D.** The patient is hypoglycemic because of deficient release of gluconeogenic amino acid precursors from muscle (low urea and glutamine, alanine and glucagon challenge tests). These results plus normal lactate and hyperketonemia eliminate deficiencies in glycogenolysis, gluconeogenesis, and lipolysis as possibilities; defective muscle glycogenolysis would not produce hypoglycemia.

12. **Answer: B.** The excessive bleeding, increased PTT, and correction of the PTT with addition of normal serum point to hemophilia. The most common types of hemophilia are hemophilia A (deficiency in clotting factor VIII) and hemophilia B (Christmas disease; caused by a deficiency in clotting factor IX). The genes encoding both of these proteins are carried on the X-chromosome, making these X-linked recessive diseases. The only disease listed above which is inherited in an X-linked manner is Fabry disease, caused by a defect in α-galactosidase A involved with degradation of glycosphingolipids.

    (**Choices C and D)** are autosomal recessive inherited disorders of sphingolipid catabolism (Gaucher and Tay-Sachs respectively) classified as lysosomal storage diseases.

    (**Choice E**) is an autosomal dominant inherited disorder (myotonic dystrophy) which displays trinucleotide repeat expansion and anticipation.

13. **Answer: D.** Niemann-Pick (Type A) disease is characterized by hepatosplenomegaly, with or without cherry-red spots in the macular region, neurologic involvement (mental retardation, failure to crawl, sit, or walk independently).

    I-cell disease (**Choice A**) is caused by a defect in Golgi-associated phosphotransferase (N-Acetylglucosamine-1-phosphotransferase), which would usually present with cardiomegaly and would not show the Zebra body inclusions typical of Niemann-Pick.

    Tay-Sachs disease (**Choice B**) is caused by a defect in hexosaminidase A. In most cases, this will present with the cherry-red spots and would not have Zebra body inclusions inside lysosomes. No hepatosplenomegaly occurs.

    (**Choice C**) describes Gaucher's disease, caused by a defect in glucocerebrosidase and would lead to "crumpled paper inclusions" inside macrophages. The features include bone pain, fractures, and infarctions along with hepatosplenomegaly. Most cases are Type 1 and don't present until late childhood or adolescence.

(**Choice E**) is a distractor. There are no relevant diseases on Step 1 associated with ganglioside synthesis.

Note: The patient in this case did not have "cherry-red spots" in the macula of the eye. Both Tay-Sachs and Niemann-Pick disease may present with cherry-red spots, but they are not specific to either disease. Similarly, their absence cannot be used to exclude either disease.

14. **Answer: A.** Needed for transport of fatty acids across the mitochondrial inner membrane.

15. **Answer: A.** Mitochondrial inner membrane.

16. **Answer: B.** Mitochondrial matrix (ketogenesis).

17. **Answer: C.** CAT-1 (CPT-1) and fatty acyl synthetase are among the few enzymes associated with the outer mitochondrial membrane.

Amino Acid Metabolism 17

Learning Objectives

- ❑ Explain information related to removal and excretion of amino groups
- ❑ Answer questions about urea cycle and urea cycle defects
- ❑ Answer questions about disorders of amino acid metabolism
- ❑ Explain information related to propionyl-CoA carboxylase and methylmalonyl-CoA
- ❑ Use knowledge of S-adenosylmethionine, folate, and cobalamin
- ❑ Explain information related to specialized products derived from amino acids
- ❑ Solve problems concerning heme synthesis
- ❑ Use knowledge of iron transport and storage
- ❑ Use knowledge of bilirubin metabolism

OVERVIEW

Protein obtained from the diet or from body protein during prolonged fasting or starvation may be used as an energy source. Body protein is catabolized primarily in muscle and in liver. Amino acids released from proteins usually lose their amino group through transamination or deamination. The carbon skeletons can be converted in the liver to glucose (glucogenic amino acids), acetyl CoA, and ketone bodies (ketogenic), or in a few cases both may be produced (glucogenic and ketogenic).

REMOVAL AND EXCRETION OF AMINO GROUPS

Excess nitrogen is eliminated from the body in the urine. The kidney adds small quantities of ammonium ion to the urine in part to regulate acid-base balance, but nitrogen is also eliminated in this process. Most excess nitrogen is converted to urea in the liver and goes through the blood to the kidney, where it is eliminated in urine.

Amino groups released by deamination reactions form ammonium ion (NH_4^+), which must not escape into the peripheral blood. An elevated concentration of ammonium ion in the blood, hyperammonemia, has toxic effects in the brain (cerebral edema, convulsions, coma, and death). Most tissues add excess nitrogen to the blood as glutamine by attaching ammonia to the γ-carboxyl group of glutamate. Muscle sends nitrogen to the liver as alanine and smaller quantities of other amino acids, in addition to glutamine. The figure below summarizes the flow of nitrogen from tissues to the liver or kidney for excretion.

Bridge to Pharmacology

Lactulose is metabolized to lactic acid in the GI tract by bacteria, which in turn covert ammonia (NH_3) to ammonium (NH_4^+), interfering with absorption and treating hyperammonemia.

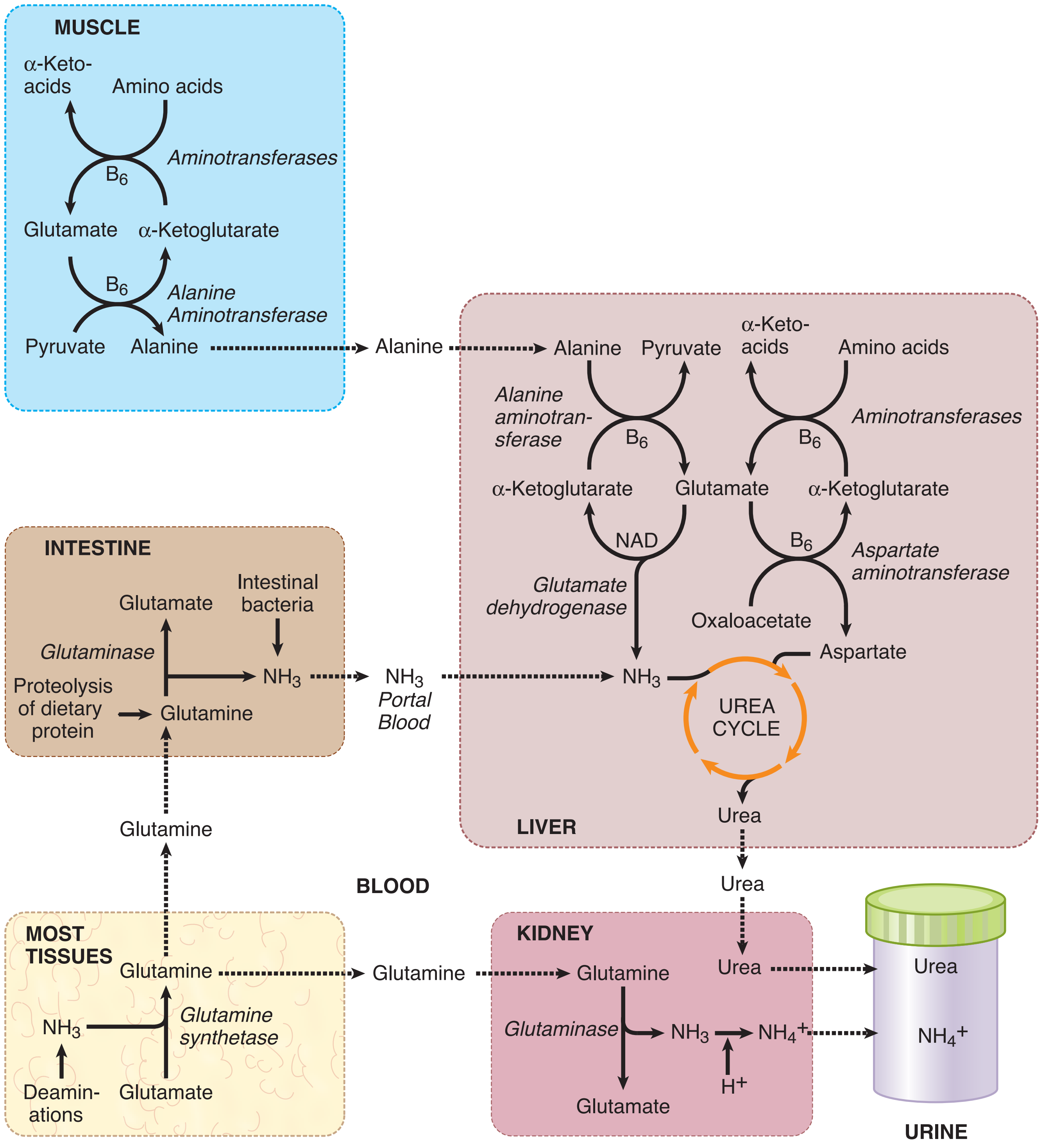


Figure I-17-1. Amino Group Removal for Elimination as Urea and Ammonia

Glutamine Synthetase

Most tissues, including muscle, have glutamine synthetase, which captures excess nitrogen by aminating glutamate to form glutamine. The reaction is irreversible. Glutamine, a relatively nontoxic substance, is the major carrier of excess nitrogen from tissues.

Glutaminase

The kidney contains glutaminase, allowing it to deaminate glutamine arriving in the blood and to eliminate the amino group as ammonium ion in urine. The reaction is irreversible. Kidney glutaminase is induced by chronic acidosis, in which excretion of ammonium may become the major defense mechanism. The liver has only small quantities of glutaminase; however, levels of the enzyme are high in the intestine where the ammonium ion from deamination can be sent directly to the liver via the portal blood and used for urea synthesis. The intestinal bacteria and glutamine from dietary protein contribute to the intestinal ammonia entering the portal blood.

Aminotransferases (Transaminases)

High-Yield

Both muscle and liver have aminotransferases, which, unlike deaminases, do not release the amino groups as free ammonium ion. This class of enzymes transfers the amino group from one carbon skeleton (an amino acid) to another (usually α-ketoglutarate, a citric acid cycle intermediate). Pyridoxal phosphate (PLP) derived from vitamin B_6 is required to mediate the transfer.

Aminotransferases are named according to the amino acid donating the amino group to α-ketoglutarate. Two important examples are alanine aminotransferase (ALT, formerly GPT) and aspartate aminotransferase (AST, formerly GOT). Although the aminotransferases are in liver and muscle, in pathologic conditions these enzymes may leak into the blood, where they are useful clinical indicators of damage to liver or muscle.

The reactions catalyzed by aminotransferases are reversible and play several roles in metabolism:

- During protein catabolism in muscle, they move the amino groups from many of the different amino acids onto glutamate, thus pooling it for transport. A portion of the glutamate may be aminated by glutamine synthetase (as in other tissues) or may transfer the amino group to pyruvate, forming alanine using the aminotransferase ALT.
- In liver, aminotransferases ALT and AST can move the amino group from alanine arriving from muscle into aspartate, a direct donor of nitrogen into the urea cycle.

Glutamate Dehydrogenase

This enzyme is found in many tissues, where it catalyzes the reversible oxidative deamination of the amino acid glutamate. It produces the citric acid cycle intermediate α-ketoglutarate, which serves as an entry point to the cycle for a group of glucogenic amino acids. Its role in urea synthesis and nitrogen removal is still controversial.

UREA CYCLE

Urea, which contains 2 nitrogens, is synthesized in the liver from aspartate and carbamoyl phosphate, which in turn is produced from ammonium ion and carbon dioxide by mitochondrial carbamoyl phosphate synthetase. This enzyme requires N-acetylglutamate as an activator. N-acetylglutamate is produced only when free amino acids are present.

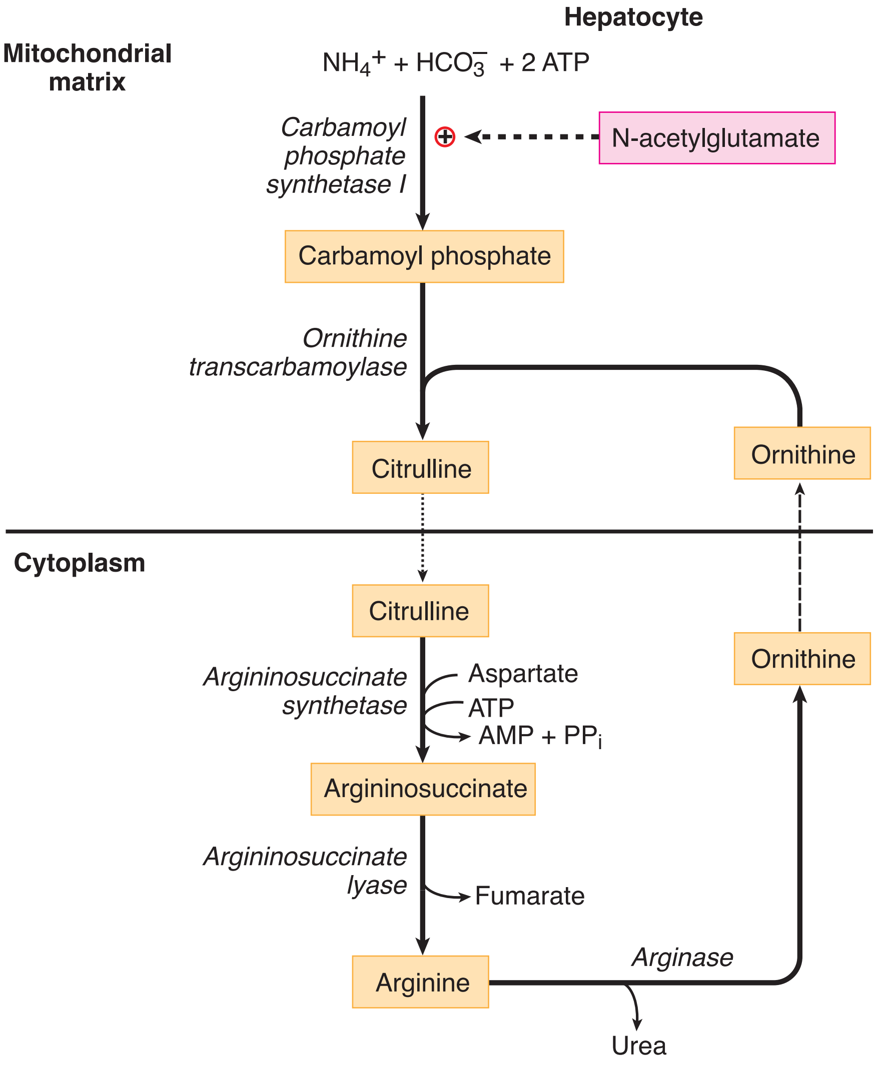


Figure I-17-2. The Urea Cycle in the Liver

The urea cycle, like the citric acid cycle, acts catalytically. Small quantities of the intermediates are sufficient to synthesize large amounts of urea from aspartate and carbamoyl phosphate. The cycle occurs partially in the mitochondria and partially in the cytoplasm.

- Citrulline enters the cytoplasm, and ornithine returns to the mitochondria.
- Carbamoyl phosphate synthetase and ornithine transcarbamoylase are mitochondrial enzymes.

- Aspartate enters the cycle in the cytoplasm and leaves the cycle (minus its amino group) as fumarate. If gluconeogenesis is active, fumarate can be converted to glucose.
- The product urea is formed in the cytoplasm and enters the blood for delivery to the kidney.

Genetic Deficiencies of the Urea Cycle

High-Yield

A combination of hyperammonemia, elevated blood glutamine, and decreased blood urea nitrogen (BUN) suggests a defect in the urea cycle. With neonatal onset, infants typically appear normal for the first 24 hours. Sometime during the 24- to 72-hour postnatal period, symptoms of lethargy, vomiting, and hyperventilation begin and, if not treated, progress to coma, respiratory failure, and death.

There are 2 deficiencies of the 2 mitochondrial enzymes in the urea cycle: carbamoyl phosphate synthetase and ornithine transcarbamoylase. They can be distinguished by an increase in orotic acid and uracil, which occurs in ornithine transcarbamoylase deficiency, but not in the deficiency of carbamoyl phosphate synthetase. Orotic acid and uracil are intermediates in pyrimidine synthesis (see Chapter 18). This pathway is stimulated by the accumulation of carbamoyl phosphate, the substrate for ornithine transcarbamoylase in the urea cycle and for aspartate transcarbamoylase in pyrimidine synthesis.

Table I-17-1. Genetic Deficiencies of Urea Synthesis

Carbamoyl Phosphate Synthetase	Ornithine Transcarbamoylase
↑ $[NH_4^+]$; hyperammonemia	↑ $[NH_4^+]$; hyperammonemia
Blood glutamine is increased	Blood glutamine is increased
BUN is decreased	BUN is decreased
No orotic aciduria Autosomal recessive	Orotic aciduria X-linked recessive
Cerebral edema	Cerebral edema
Lethargy, convulsions, coma, death	Lethargy, convulsions, coma, death

These conditions can be treated with a low protein diet and administration of sodium benzoate or phenylpyruvate to provide an alternative route for capturing and excreting excess nitrogen.

DISORDERS OF AMINO ACID METABOLISM

The figure below presents a diagram of pathways in which selected amino acids are converted to citric acid cycle intermediates (and glucose) or to acetyl-CoA (and ketones). Important genetic deficiencies are identified.

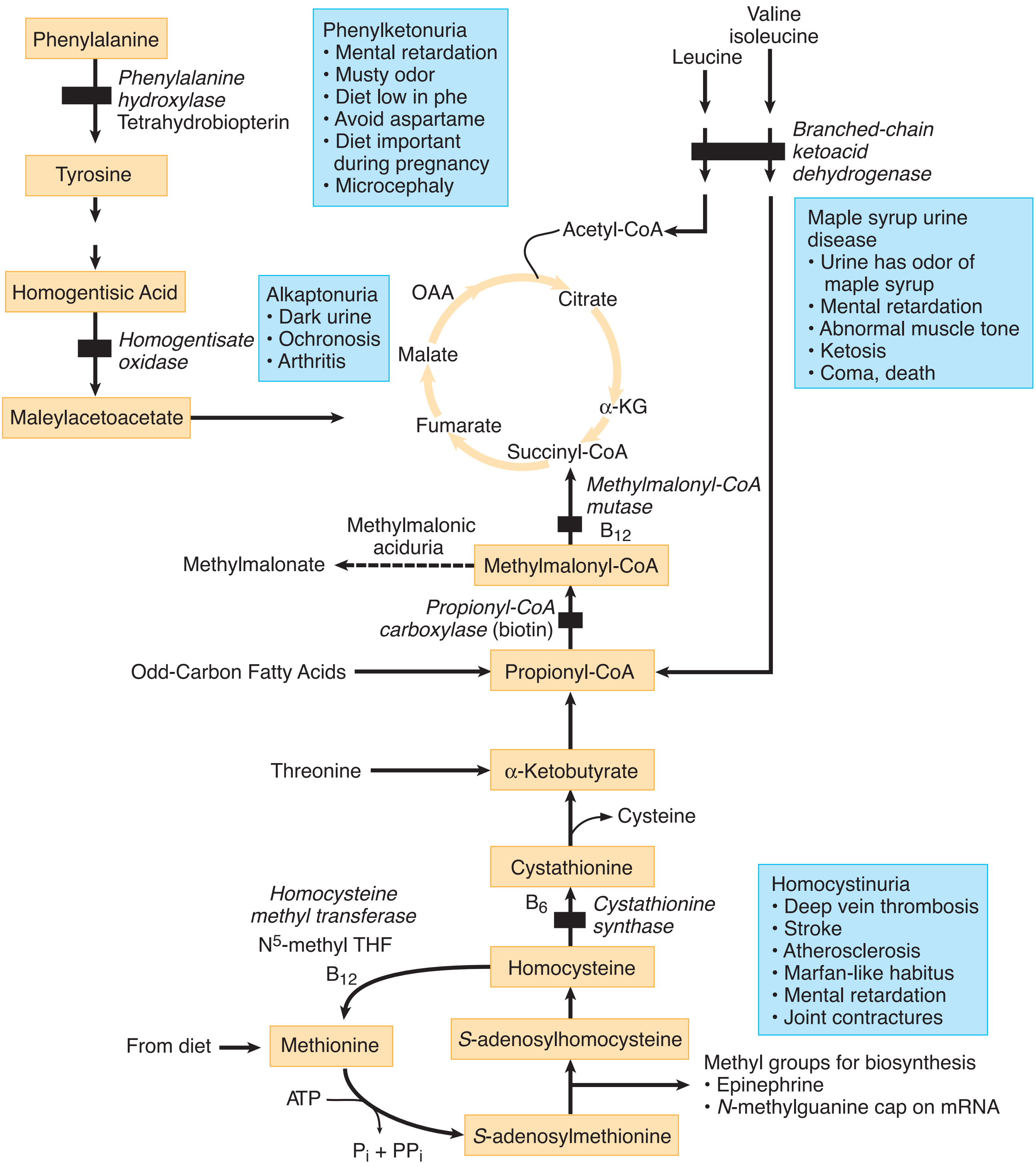


Figure I-17-3. Genetic Deficiencies of Amino Acid Metabolism

Phenylalanine Hydroxylase Deficiency (Phenylketonuria)

High-Yield

Infants with classic phenylketonuria (PKU) are normal at birth but if untreated show slow development, severe mental retardation, autistic symptoms, and loss of motor control. Children may have pale skin and white-blonde hair. The neurotoxic effects relate to high levels of phenylalanine and not to the phenylketones from which the name of the disease derives. Infants are routinely screened a few days after birth for blood phenylalanine level. Treatment is a lifelong semi-synthetic diet restricted in phenylalanine (small quantities are necessary because it is an essential amino acid).

Women with PKU who become pregnant must be especially careful about the phenylalanine level in their blood so as not to adversely affect neurologic development in the fetus. Infants whose phenylketonuric mothers have not maintained adequate metabolic control during pregnancy have a high risk for mental retardation (although less profound than in a child with untreated PKU), microcephaly, and low birth weight.

Note

Aspartame (N-aspartylphenylalanine methyl ester), a widely used artificial sweetener, must be **strictly avoided by phenylketonurics**.

Bridge to Medical Genetics

There are >100 known mutations in the gene for phenylalanine hydroxylase, causing PKU. This is an example of allelic heterogeneity.

Albinism

High-Yield

Albinism (1:15,000) is a group of conditions in which then normal conversion of tyrosine to melanin is altered. The most severe form is a deficiency of tyrosinase, causing an absence of pigment in the skin, hair, and eyes.

Homogentisate Oxidase Deficiency (Alcaptonuria)

High-Yield

Accumulation of homogentisic acid in the blood causes its excretion in urine, after which it gradually darkens upon exposure to air. This sign of alcaptonuria is not present in all patients with the enzyme deficiency. The dark pigment also accumulates over years in the cartilage (ochronosis) and may be seen in the sclera of the eye, in ear cartilage and patients develop arthritis in adulthood, usually beginning in the third decade. Treatment is targeted to managing the symptoms.

Branched-Chain Ketoacid Dehydrogenase Deficiency (Maple Syrup Urine Disease)

High-Yield

Branched-chain ketoacid dehydrogenase, an enzyme similar to α-ketoglutarate dehydrogenase (thiamine, lipoic acid, CoA, FAD, NAD^+), metabolizes branched-chain ketoacids produced from their cognate amino acids, valine, leucine, and isoleucine. In the classic form of the disease, infants are normal for the first few days of life, after which they become progressively lethargic, lose weight, and have alternating episodes of hypertonia and hypotonia, and the urine develops a characteristic odor of maple syrup. Ketosis, coma, and death ensue if not treated. Treatment requires restricting dietary valine, leucine, and isoleucine.

Propionyl-CoA Carboxylase and Methylmalonyl-CoA Mutase Deficiencies

High-Yield

Valine, methionine, isoleucine, and threonine are all metabolized through the propionic acid pathway (also used for odd-carbon fatty acids). Deficiency of either enzyme results in neonatal ketoacidosis from failure to metabolize ketoacids produced from these 4 amino acids. The deficiencies may be

Note

- **Propionyl CoA carboxylase deficiency** involves an accumulation of propionic acid, methyl citrate, and hydroxypropionic acid.
- **Methylmalonyl CoA mutase deficiency** involves an accumulation of methylmalonic acid.

distinguished based on whether methylmalonic aciduria is present (methylmalonyl CoA mutase deficiency) or by the presence of methyl citrate and hydroxypropionate (propionyl CoA carboxylase deficiency). A diet low in protein or a semisynthetic diet with low amounts of valine, methionine, isoleucine, and threonine is used to treat both deficiencies.

Homocystinemia/Homocystinuria

Accumulation of homocystine in blood is associated with cardiovascular disease; deep vein thrombosis, thromboembolism, and stroke; dislocation of the lens (ectopic lens); and mental retardation. Homocystine is a disulfide dimer of homocysteine. Homocystinemia caused by an enzyme deficiency is a rare, but severe, condition in which atherosclerosis in childhood is a prominent finding. These children often have myocardial infarctions before 20 years of age. All patients excrete high levels of homocystine in the urine. Treatment includes a diet low in methionine. The major enzyme deficiency producing homocystinemia is that of cystathionine synthase:

> A 5-year-old girl was brought to her pediatrician because she had difficulty with her vision and seemed to be slow in her mental and physical development since birth. The physician noted that the girl had abnormally long, "spidery" fingers and a downward dislocation of the right lens of her eye. Further examination revealed a deep vein thrombosis. A laboratory examination of her blood indicated increased methionine. She also had increased urinary excretion of homocystine, indicated by a cyanide-nitroprusside test. The parents were advised to restrict methionine to low levels and supplement folate, vitamin B_{12}, and vitamin B_6 in the girl's diet.

Homocystinuria caused by a genetic defect in the enzyme cystathionine synthase is rare and can present similarly to Marfan syndrome. The latter is a defect in the fibrillin gene, resulting in tall stature, long fingers and toes, lens dislocation, and a tendency toward aortic wall ruptures. In cystathionine synthase deficiency subluxation of the lens is downward and inward. In Marfan syndrome subluxation of the lens is upward and outward. Cystathionine synthase deficiency results in the accumulation of homocysteine and methionine and their spillage into blood and urine. Two molecules of homocysteine can oxidize to the disulfide-crosslinked homocystine. Many patients with homocystinuria who have partial activity of cystathionine synthase respond well to pyridoxine administration. If left untreated, patients will usually succumb to myocardial infarction, stroke, or pulmonary embolism.

Homocystinemia from Vitamin Deficiencies

High-Yield

Vitamin deficiencies may produce a more mild form of homocystinemia. Mild homocystinemia is associated with increased risk for atherosclerosis, deep vein thrombosis, and stroke. The vitamin deficiencies causing homocystinemia include:

- Folate deficiency: recommended dietary intake of folate has been increased (also protects against neural tube defects in the fetus), and additional folate is now added to flour (bread, pasta, and other products made from flour)
- Vitamin B12
- Vitamin B6

Recall Question

Which of the following results from a deficiency of ornithine transcarbamoylase but not of carbamoyl phosphate synthetase?

A. Cerebral edema

B. Decreased BUN

C. Hyperammonemia

D. Increased blood glutamine

E. Orotic aciduria

Answer: E

S-ADENOSYLMETHIONINE, FOLATE, AND COBALAMIN

One-Carbon Units in Biochemical Reactions

One-carbon units in different oxidation states are required in the pathways producing purines, thymidine, and many other compounds. When a biochemical reaction requires a methyl group (methylation), *S*-adenosylmethionine (SAM) is generally the methyl donor.

If a 1-carbon unit in another oxidation state is required (methylene, methenyl, formyl), tetrahydrofolate (THF) typically serves as its donor.

S-Adenosylmethionine

Important pathways requiring SAM include synthesis of epinephrine and of the 7-methylguanine cap on eukaryotic mRNA. After donating the methyl group, SAM is converted to homocysteine and remethylated in a reaction catalyzed by *N*-methyl THF–homocysteine methyltransferase requiring both vitamin B_{12} and *N*-methyl-THF. The methionine produced is once again used to make SAM.

Tetrahydrofolate

High-Yield

THF is formed from the vitamin folate through 2 reductions involving NADPH and catalyzed by dihydrofolate reductase. It picks up a 1-carbon unit from a variety of donors and enters the active 1-carbon pool. Important pathways requiring forms of THF from this pool include the synthesis of all purines and thymidine, which in turn are used for DNA and RNA synthesis during cell growth and division.

Megaloblastic anemia results from insufficient active THF to support cell division in the bone marrow. Methotrexate inhibits DHF reductase, making it a useful antineoplastic drug. Folate deficiencies may be seen during pregnancy and in alcoholism.

Additional folate may be stored as the highly reduced N^5-methyl-THF. This form is referred to as the storage pool as there is only one known enzyme that uses it, and in turn moves it back into the active pool. This enzyme is *N*-methyl THF-homocysteine methyltransferase, which also requires vitamin B12 and is involved in regenerating SAM as a methyl donor for reactions.

Note

THB (BH4) is necessary for tyrosine hydroxylase, phenylalanine hydroxylase, and tryptophan hydroxylase (serotonin synthesis) and is regenerated by dihydropteridine reductase.

Clinical Correlate

Parkinson's disease is caused by loss of dopaminergic neurons in the substantia nigra. It is treated with levodopa (L-dopa).

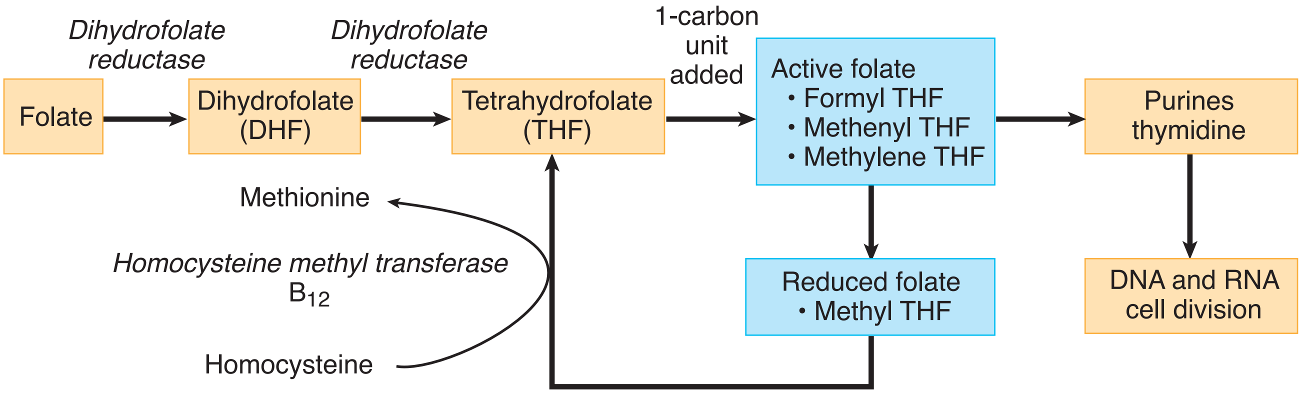


Figure I-17-4. Folate Metabolism

Cobalamin

High-Yield

The vitamin cobalamin (vitamin B12) is reduced and activated in the body to 2 forms: adenosylcobalamin (used by methylmalonyl-CoA mutase) and methylcobalamin (formed from N^5-methyl-THF in the *N*-methyl THF-homocysteine methyltransferase reaction). These are the only 2 enzymes which use B12 (other than the enzymes that reduce and add an adenosyl group to it).

Cobalamin deficiency can result in the following:

- Secondary deficiency of active THF, by preventing its release from the storage pool through the N-methyl THF-homocysteine methyltransferase reaction (thus also results in megaloblastic anemia)
- Progressive peripheral neuropathy
- Pernicious anemia (most likely reason), a failure to absorb vitamin B12 in the absence of intrinsic factor from parietal cells
  - B12 absorption also decreases with aging and in individuals with chronic pancreatitis.
  - B12 absorption can also decrease (less commonly) with a long-term, completely vegetarian diet (plants don't contain B12) or infection with Diphyllobothrium latum, a parasite found in raw fish (excess B12 is stored in the body, so deficiencies develop slowly)

Treating a cobalamin deficiency with folate corrects the megaloblastic anemia but does not halt the neuropathy.

Table I-17-2. Folate versus Vitamin B12 Deficiency

Folate Deficiency	Vitamin B12 (Cobalamin) Deficiency
Megaloblastic anemia • Macrocytic • MCV >100 femtoliters (fL) • PMN nucleus more than 5 lobes Homocysteinemia with risk for cardiovascular disease	Megaloblastic anemia • Macrocytic • MCV >100 femtoliters (fL) • PMN nucleus more than 5 lobes Homocysteinemia with risk for cardiovascular disease Methylmalonic aciduria Progressive peripheral neuropathy
Deficiency develops in 3–4 months Risk factors for deficiency: • Pregnancy (neural tube defects in fetus may result) • Alcoholism • Severe malnutrition • Gastric or terminal ileum resection	Deficiency develops in years Risk factors for deficiency: • Pernicious anemia • Gastric resection • Chronic pancreatitis • Severe malnutrition • Vegan • Infection with *D. latum* • Aging • Bacterial overgrowth of the terminal ileum • *H. pylori* infection

Bridge to Pathology

Vitamin B12 deficiency causes demyelination of the posterior columns and lateral corticospinal tracts in the spinal cord.

SPECIALIZED PRODUCTS DERIVED FROM AMINO ACIDS

Table I-17-3. Products of Amino Acids

Amino Acid	Products
Tyrosine	Thyroid hormones T_3 and T_4 Melanin Catecholamines
Tryptophan	Serotonin NAD, NADP
Arginine	Nitric oxide (NO)
Glutamate	γ-Aminobutyric acid (GABA)
Histidine	Histamine

HEME SYNTHESIS

Heme synthesis occurs in almost all tissues because heme proteins include not only hemoglobin and myoglobin but all the cytochromes (electron transport chain, cytochrome P-450, cytochrome b_5), as well as the enzymes catalase, peroxidase, and the soluble guanylate cyclase stimulated by nitric oxide. The pathway producing heme, as shown below, is controlled independently in different tissues. In liver, the rate-limiting enzyme δ-aminolevulinate synthase (ALA) is repressed by heme.

Note

Compounds with an "-ogen" suffix, such as urobilinogen, are colorless substances. In the presence of oxygen, they spontaneously oxidize, forming a conjugated double-bond network in the compounds. These oxidized compounds are highly colored substances and have an "-in" suffix (e.g., porphobilin, urobilin).

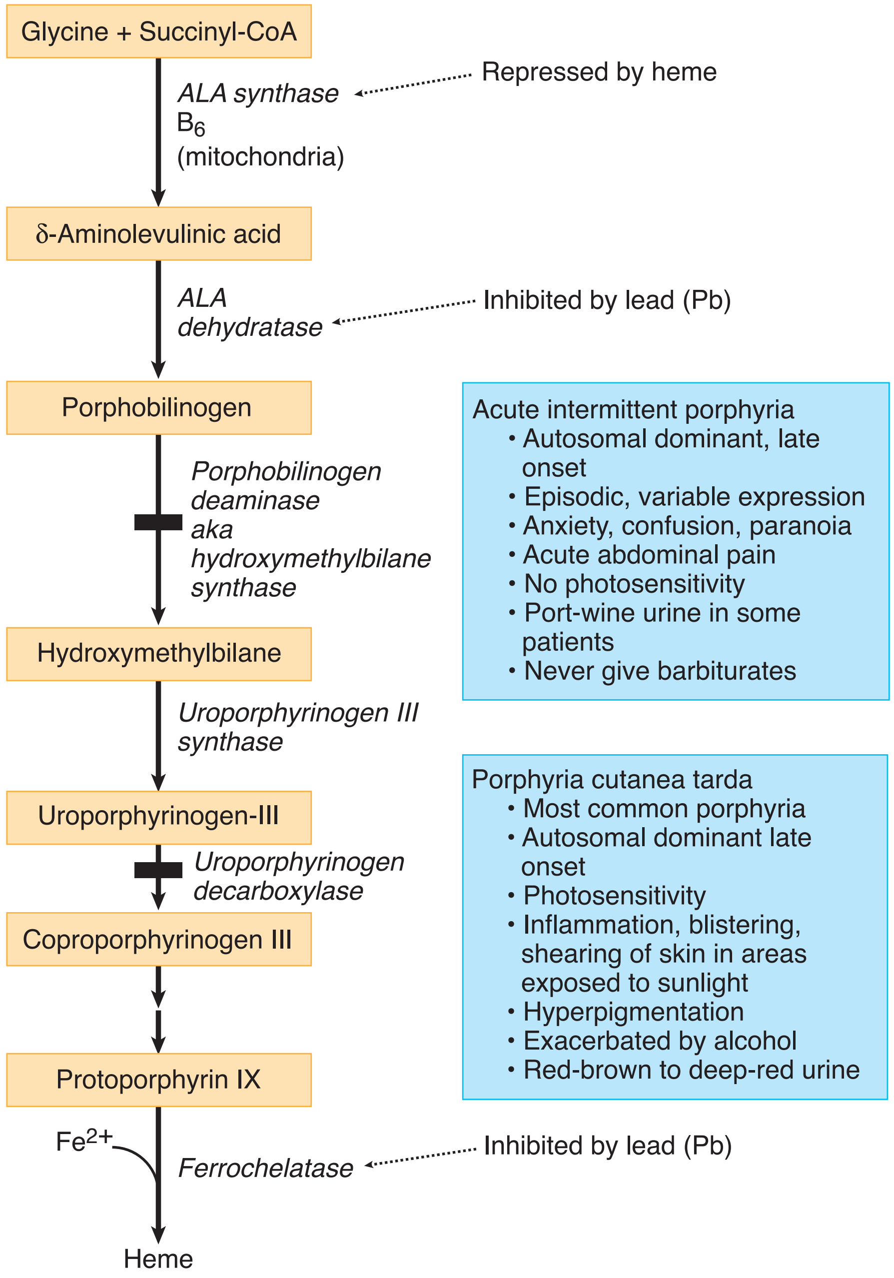


Figure I-17-5. Heme Synthesis

Acute Intermittent Porphyria: Porphobilinogen Deaminase (Hydroxymethylbilane Synthase) Deficiency

High-Yield

This late-onset autosomal dominant disease exhibits variable expression. Many heterozygotes remain symptom-free throughout their lives. Signs and symptoms, when present, include:

- Abdominal pain, often resulting in multiple laparoscopies (scars on abdomen)
- Anxiety, paranoia, and depression

- Paralysis
- Motor, sensory or autonomic neuropathy
- Weakness
- Excretion of ALA (δ-aminolevulinic) and PBG (porphobilinogen) during episodes
- In severe cases, dark port-wine color to urine on standing

Some of these individuals are incorrectly diagnosed and placed in psychiatric institutions. Episodes may be induced by hormonal changes and by many drugs, including barbiturates.

Other Porphyrias

High-Yield

Deficiencies of other enzymes in the heme pathway produce porphyrias in which photosensitivity is a common finding. Chronic inflammation to overt blistering and shearing in exposed areas of the skin characterize these porphyrias. The most common is porphyria cutanea tarda (deficiency of uroporphyrinogen decarboxylase), an autosomal dominant condition with late onset. β-Carotene is often administered to porphryia patients with photosensitivity to reduce the production of reactive oxygen species.

Porphyria cutanea tarda

A 35-year-old man was becoming very sensitive to sunlight and often detected persistent rashes and blisters throughout areas of his body that were exposed to the sun. He also observed that drinking excessive alcohol with his friends after softball games worsened the incidence of the recurrent blisters and sunburns. He became even more concerned after he noticed his urine became a red-brown tint if he did not flush the toilet.

Porphyria cutanea tarda is an adult-onset hepatic porphyria in which hepatocytes are unable to decarboxylate uroporphyrinogen in heme synthesis. The uroporphyrin spills out of the liver and eventually into urine, giving rise to the characteristic red-wine urine if it is allowed to stand, a hallmark of porphyrias. Hepatotoxic substances, such as excessive alcohol or iron deposits, can exacerbate the disease. Skin lesions are related to high circulating levels of porphyrins.

Vitamin B6 Deficiency

High-Yield

ALA synthase, the rate-limiting enzyme, requires pyridoxine (vitamin B6). Deficiency of pyridoxine is associated with isoniazid therapy for tuberculosis and may cause sideroblastic anemia with ringed sideroblasts.

Iron Deficiency

High-Yield

The last enzyme in the pathway, heme synthase (ferrochelatase), introduces the Fe^{2+} into the heme ring. Deficiency of iron produces a microcytic hypochromic anemia.

Bridge to Pharmacology

Barbiturates are hydroxylated by the microsomal cytochrome P-450 system in the liver to facilitate their efficient elimination from the body. Administration of the barbiturates results in stimulation of cytochrome P-450 synthesis, which in turn reduces heme levels. The reduction in heme lessens the repression of ALA synthase, causing more porphyrin precursor synthesis. In porphyrias, the indirect production of more precursors by the barbiturates exacerbates the disease.

Lead Poisoning

High-Yield

Lead inactivates many enzymes including ALA dehydrase and ferrochelatase (heme synthase), and can produce a microcytic sideroblastic anemia with ringed sideroblasts in the bone marrow. Other symptoms include:

- Coarse basophilic stippling of erythrocytes
- Headache, nausea, memory loss
- Abdominal pain, diarrhea (lead colic)
- Lead lines in gums
- Lead deposits in abdomen and epiphyses of bone seen on radiograph
- Neuropathy (claw hand, wrist-drop)
- Increased urinary ALA
- Increased free erythrocyte protoporphyrin

Vitamin B6 deficiency, iron deficiency, and lead poisoning all can cause anemia.

Table I-17-4. Vitamin B6 Deficiency, Iron Deficiency, and Lead Poisoning

Vitamin B_6 (Pyridoxine) Deficiency	Iron Deficiency	Lead Poisoning
Microcytic	Microcytic	Microcytic Coarse basophilic stippling in erythrocyte
Ringed sideroblasts in bone marrow		Ringed sideroblasts in bone marrow
Protoporphyrin: ↓	Protoporphyrin: ↑	Protoporphyrin: ↑
δ-ALA: ↓	δ-ALA: Normal	δ-ALA: ↑
Ferritin: ↑	Ferritin: ↓	Ferritin: ↑
Serum iron: ↑	Serum iron: ↓	Serum iron: ↑
Isoniazid for tuberculosis	Dietary iron insufficient to compensate for normal loss	Lead paint Pottery glaze Batteries (Diagnose by measuring blood lead level)

IRON TRANSPORT AND STORAGE

Iron (Fe^{3+}) released from hemoglobin in the histiocytes is bound to ferritin and then transported in the blood by transferrin, which can deliver it to tissues for synthesis of heme. Important proteins in this context are:

- Ferroxidase (also known as ceruloplasmin, a Cu^{2+} protein) oxidizes Fe^{2+} to Fe^{3+} for transport and storage.
- Transferrin carries Fe^{3+} in blood.

Clinical Correlate

The failure of ferrochelatase to insert Fe^{2+} into protoporphyrin IX to form heme, such as in lead poisoning or iron deficiency anemia, results in the nonenzymatic insertion of Zn^{2+} to form zinc-protoporphyrin. This complex is extremely fluorescent and is easily detected.

Note

Anemia is an important topic on the exam. Compare and contrast all anemias (differential diagnosis).

Bridge to Pathology

Hemochromatosis is an inherited, autosomal recessive disease (prevalence 1/200) generally seen in men age >40 and in older women. The disease is characterized by a daily intestinal absorption of 2–3 mg of iron compared with the normal 1 mg. Over 20–30 years, the disease results in levels of 20–30 grams of iron in the body (normal 4 grams). Hemosiderin deposits are found in the liver, pancreas, skin, and joints.

- Ferritin itself oxidizes Fe^{2+} to Fe^{3+} for storage of normal amounts of Fe^{3+} in tissues. Loss of iron from the body is accomplished by bleeding and shedding epithelial cells of the mucosa and skin. The body has no mechanism for excreting iron, so controlling its absorption into the mucosal cells is crucial. No other nutrient is regulated in this manner.
- Hemosiderin binds excess Fe^{3+} to prevent escape of free Fe^{3+} into the blood, where it is toxic.

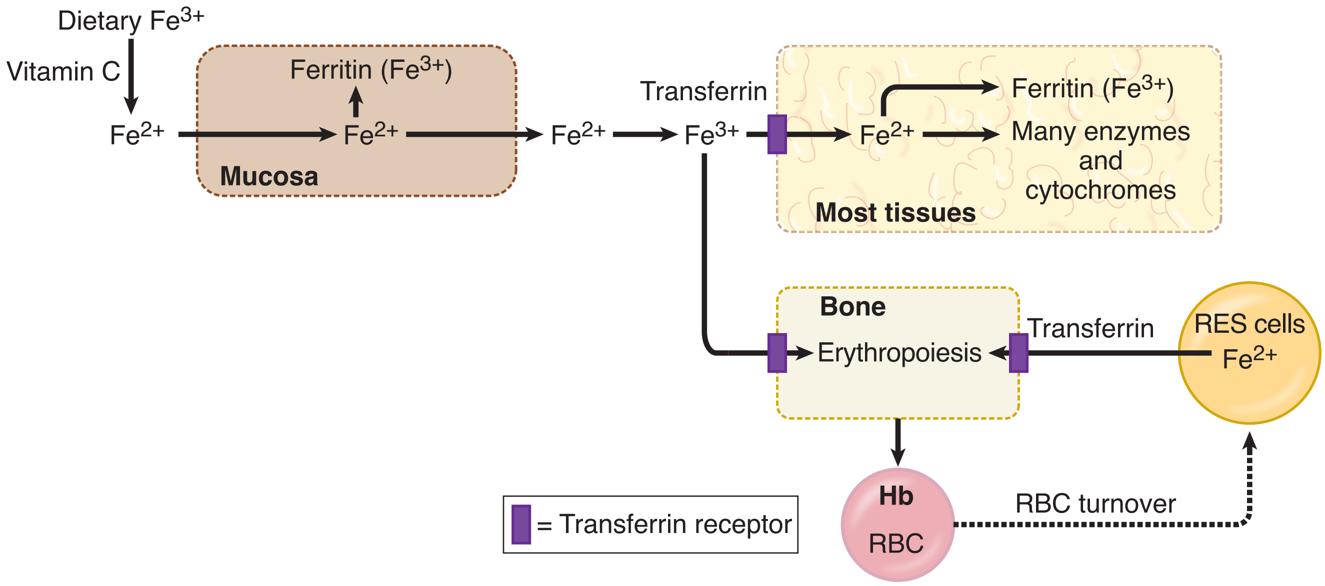


Figure I-17-6. Iron Metabolism

BILIRUBIN METABOLISM

Subsequent to lysis of older erythrocytes in the spleen, heme released from hemoglobin is converted to bilirubin in the histiocytes.

- Bilirubin is not water-soluble and is therefore transported in the blood attached to serum albumin.
- Hepatocytes conjugate bilirubin with glucuronic acid, increasing its water solubility.
- Conjugated bilirubin is secreted into the bile.
- Intestinal bacteria convert conjugated bilirubin into urobilinogen.
- A portion of the urobilinogen is further converted to bile pigments (stercobilin) and excreted in the feces, producing their characteristic red-brown color. Bile duct obstruction results in clay-colored stools.
- Some of the urobilinogen is converted to urobilin (yellow) and excreted in urine.

Clinical Correlate

Excessive RBC destruction in hemolytic anemia results in excessive conversion of bilirubin to urobilinogen in the intestine. Higher-than-normal absorption of the urobilinogen and its subsequent excretion in the urine results in a deeper-colored urine.

Clinical Correlate

At very high levels, lipid-soluble bilirubin may cross the blood-brain barrier and precipitate in the basal ganglia, causing irreversible brain damage (kernicterus).

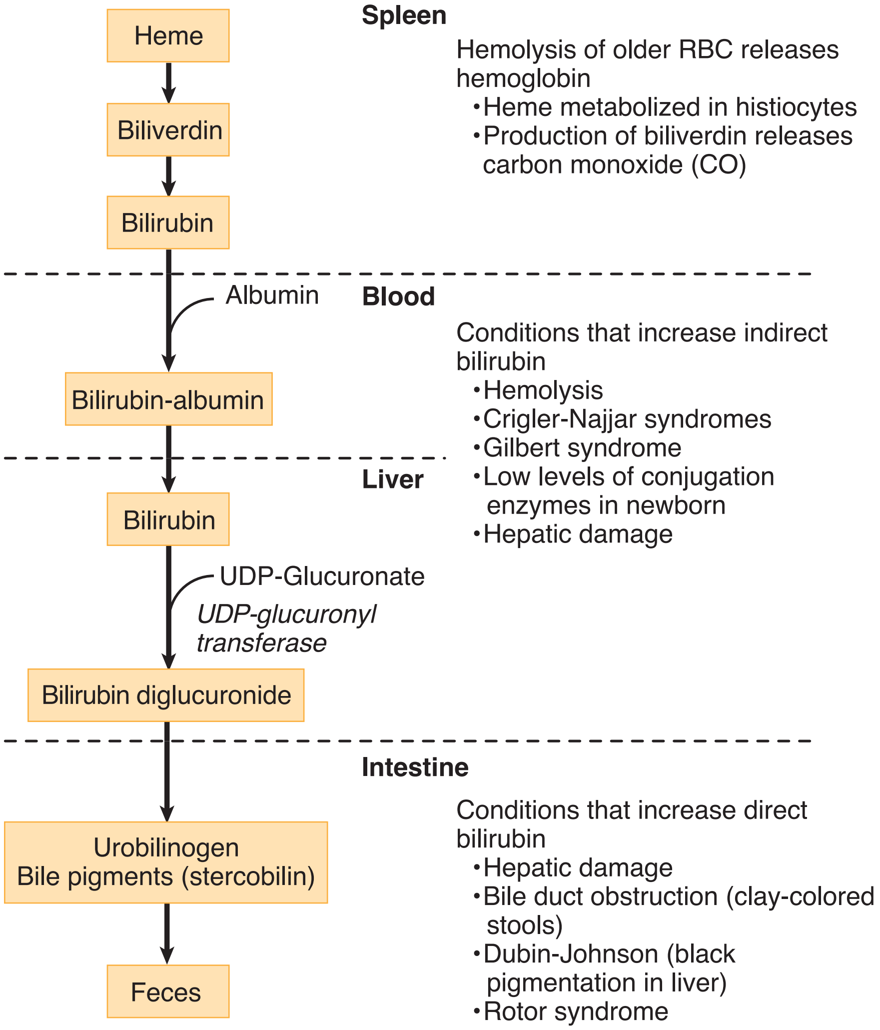


Figure I-17-7. Heme Catabolism and Bilirubin

Recall Question

Which substance is the human body unable to excrete?

A. Biotin

B. Cobalamine

C. Iron

D. Niacin

Answer: C

Bilirubin and Jaundice

High-Yield

Jaundice (yellow color of skin, whites of the eyes) may occur when blood levels of bilirubin exceed normal (icterus). It may be characterized by an increase in unconjugated (indirect) bilirubin, conjugated (direct) bilirubin, or both. Accumulation of bilirubin (usually unconjugated) in the brain (kernicterus) may result in death.

When conjugated bilirubin increases, it may be excreted, giving a deep yellow-red color to the urine.

Examples of conditions associated with increased bilirubin and jaundice include hemolytic crisis, UDP-glucuronyl transferase deficiency, hepatic damage, and bile duct occlusion.

Hemolytic crisis

With severe hemolysis, more bilirubin is released into the blood than can be transported on albumin and conjugated in the liver. Unconjugated and total bilirubin increase and may produce jaundice and kernicterus. Examples include:

- Episode of hemolysis in G6PDH deficiency
- Sickle cell crisis
- Rh disease of newborn

Hemolytic crisis may be confirmed by low hemoglobin and elevated reticulocyte counts.

UDP-glucuronyl transferase deficiency

When bilirubin conjugation is low because of genetic or functional deficiency of the glucuronyl transferase system, unconjugated and total bilirubin increase. Examples include:

- Crigler-Najjar syndromes (types I and II)
- Gilbert syndrome
- Physiologic jaundice in the newborn, especially premature infants (enzymes may not be fully induced)

Hepatic damage

Viral hepatitis or cirrhosis produces an increase in both direct and indirect bilirubin. Aminotransferase levels will also be elevated.

- Alcoholic liver disease, AST increases more than ALT
- Viral hepatitis, ALT increases more than AST

Bile duct occlusion

Occlusion of the bile duct (gallstone, primary biliary cirrhosis, pancreatic cancer) prevents conjugated bilirubin from leaving the liver. Conjugated bilirubin increases in blood and may also appear in urine. Feces are light-colored.

Review Questions

Select the ONE best answer.

1. Which enzymes are responsible for producing the direct donors of nitrogen into the pathway producing urea?

   A. Arginase and argininosuccinate lyase
   B. Xanthine oxidase and guanine deaminase
   C. Glutamate dehydrogenase and glutaminase
   D. Argininosuccinate synthetase and ornithine transcarbamoylase
   E. Aspartate aminotransferase and carbamoyl phosphate synthetase

2. Two days after a full-term normal delivery, a neonate begins to hyperventilate, develops hypothermia and cerebral edema, and becomes comatose. Urinalysis reveals high levels of glutamine and orotic acid. BUN is below normal. Which enzyme is most likely to be deficient in this child?

   A. Cytoplasmic glutaminase
   B. Cytoplasmic carbamoyl phosphate synthetase
   C. Cytoplasmic orotidylate decarboxylase
   D. Mitochondrial carbamoyl phosphate synthetase
   E. Mitochondrial ornithine transcarbamoylase

Items 3 and 4

A 49-year-old man with a rare recessive condition is at high risk for deep vein thrombosis and stroke and has had replacement of ectopic lenses. He has a normal hematocrit and no evidence of megaloblastic anemia.

3. A mutation in the gene encoding which of the following is most likely to cause this disease?

   A. Cystathionine synthase
   B. Homocysteine methyltransferase
   C. Fibrillin
   D. Lysyl oxidase
   E. Branched chain α-ketoacid dehydrogenase

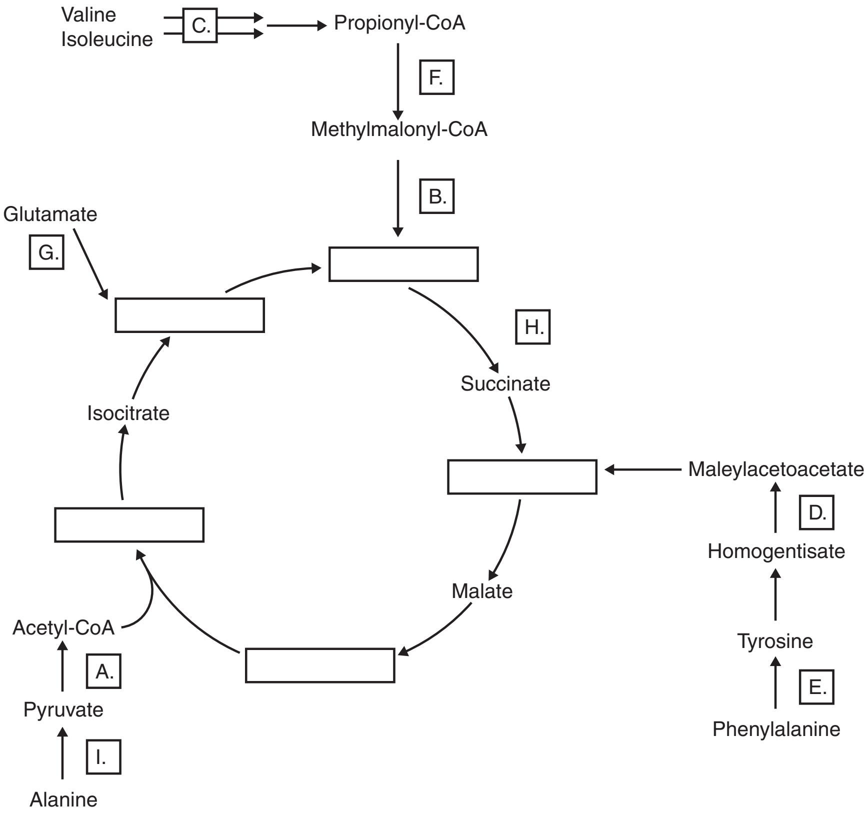
4. Amino acid analysis of this patient's plasma would most likely reveal an abnormally elevated level of

   A. lysine
   B. leucine
   C. methionine
   D. ornithine
   E. cysteine

5. A 56-year-old man with a history of genetic disease undergoes hip replacement surgery for arthritis. During the operation the surgeon notes a dark pigmentation (ochronosis) in the cartilage. His ochronotic arthritis is most likely caused by oxidation and polymerization of excess tissue

   A. homogentisic acid
   B. orotic acid
   C. methylmalonic acid
   D. uric acid
   E. ascorbic acid

Items 6–8



For each of the conditions below, link the missing substrate or enzyme.

6. A 9-week-old boy, healthy at birth, begins to develop symptoms of ketoacidosis, vomiting, lethargy, seizures and hypertonia. Urine has characteristic odor of maple syrup.

7. A child with white-blond hair, blue eyes, and pale complexion is on a special diet in which one of the essential amino acids is severely restricted. He has been told to avoid foods artificially sweetened with aspartame.

8. A chronically ill patient on long-term (home) parenteral nutrition develops metabolic acidosis, a grayish pallor, scaly dermatitis, and alopecia (hair loss). These symptoms subside upon addition of the B vitamin biotin to the alimentation fluid.

9. A woman 7 months pregnant with her first child develops anemia. Laboratory evaluation indicates an increased mean cell volume (MVC), hypersegmented neutrophils, and altered morphology of several other cell types. The most likely underlying cause of this woman's anemia is

   A. folate deficiency
   B. iron deficiency
   C. glucose 6-phosphate dehydrogenase deficiency
   D. cyanocobalamin (B_{12}) deficiency
   E. lead poisoning

Items 10 and 11

A 64-year-old woman is seen by a hematologist for evaluation of a macrocytic anemia. The woman was severely malnourished. Both homocysteine and methylmalonate were elevated in her blood and urine, and the transketolase level in her erythrocytes was below normal.

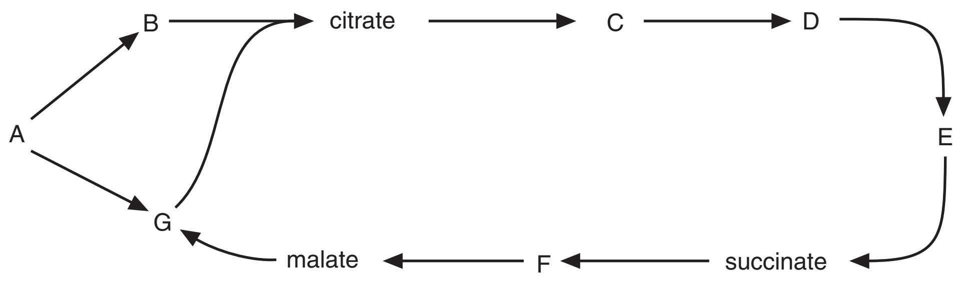
10. What is the best evidence cited that the anemia is due to a primary deficiency of cyanocobalamin (B_{12})?

   A. Macrocytic anemia
   B. Elevated methylmalonate
   C. Low transketolase activity
   D. Elevated homocysteine
   E. Severe malnutrition

11. In response to a B_{12} deficiency, which of the additional conditions may develop in this patient if she is not treated?

   A. Progressive peripheral neuropathy
   B. Gout
   C. Wernicke-Korsakoff
   D. Destruction of parietal cells
   E. Bleeding gums and loose teeth

Items 12–15



Link the following to the letters in the cycle.

12. Obligate activator of hepatic pyruvate carboxylase in the postabsorptive state.

13. Product formed by argininosuccinate lyase during urea synthesis.

14. Substrate and energy source for synthesis of δ-aminolevulinate in the heme pathway.

15. Converted to glutamate in a reaction requiring the coenzyme form of pyridoxine (B_6)

16. A 62-year-old man being treated for tuberculosis develops a microcytic, hypochromic anemia. Ferritin levels are increased, and marked sideroblastosis is present. A decrease in which of the following enzyme activities is most directly responsible for the anemia in this man?

    A. Cytochrome oxidase
    B. Cytochrome P_{450} oxidase
    C. Pyruvate kinase
    D. δ-Aminolevulinate synthase
    E. Lysyl oxidase

17. A 48-year-old man developed abdominal colic, muscle pain, and fatigue. Following a 3-week hospitalization, acute intermittent porphyria was initially diagnosed based on a high level of urinary δ-aminolevulinic acid. Subsequent analysis of the patient's circulating red blood cells revealed that 70% contained elevated levels of zinc protoporphyrin, and the diagnosis was corrected. The correct diagnosis is most likely to be

    A. protoporphyria
    B. congenital erythropoietic porphyria
    C. lead poisoning
    D. barbiturate addiction
    E. iron deficiency

18. A 3-week-old infant has been having intermittent vomiting and convulsions. She also has had episodes of screaming and hyperventilation. The infant has been lethargic between episodes. Tests reveal an expanded abdomen, and blood values show decreased citrulline amounts as well as a decreased BUN. What other clinical outcomes would be expected in this infant?

    A. Decreased blood pH and uric acid crystals in urine
    B. Decreased blood pH and increased lactic acid in blood
    C. Increased blood glutamine and increased orotic acid in urine
    D. Increased blood ammonia and increased urea in urine
    E. Megaloblastic anemia and increased methylmalonic acid in blood

19. A 69-year-old male presents to his family physician with a complaint of recent onset difficulty in performing activities of daily living. He is a retired factory worker who last worked 4 years ago. Upon questioning, his spouse reveals that he "hasn't been able to get around the way he used to." Physical examination reveals a well-nourished 69-year-old man who walks with an exaggerated kyphosis. His gait appears to be quite slow and wide-based. He also appears to have a resting tremor. The appropriate management of his case would target which of the following?

    A. Amino acid degradation
    B. Catecholamine synthesis
    C. Ganglioside degradation
    D. Prostaglandin synthesis
    E. Sphingolipid degradation

Answers

1. **Answer: E.** Aspartate is produced by AST and carbamoyl phosphate by CPS-I.

2. **Answer: E.** Given these symptoms, the defect is in the urea cycle and the elevated orotate suggests deficiency of ornithine transcarbamoylase.

3. **Answer: A.** Homocysteine, the substrate for the enzyme, accumulates increasing the risk of deep vein thrombosis and disrupting the normal crosslinking of fibrillin. Deficiency of homocysteine methyltransferase would cause homocystinuria, but would also predispose to megaloblastic anemia.

4. **Answer: C.** Only methionine is degraded via the homocysteine/cystathionine pathway and would be elevated in the plasma of a cystathionine synthase–deficient patient via activation of homocysteine methyltransferase by excess substrate.

5. **Answer: A.** Adults with alcaptonuria show a high prevalence of ochronotic arthritis due to deficiency of homogentisate oxidase.

6. **Answer: C.** Maple syrup urine disease; substrates are branched chain α-ketoacids derived from the branched chain amino acids.

7. **Answer: E.** The child has PKU; aspartame contains phenylalanine. These children may be blond, blue-eyed, and pale complected because of deficient melanin production from tyrosine.

8. **Answer: F.** The only biotin-dependent reaction in the diagram. The enzyme is propionyl-CoA carboxylase.

9. **Answer: A.** Pregnant woman with megaloblastic anemia and elevated serum homocysteine strongly suggests folate deficiency. Iron deficiency presents as microcytic, hypochromic anemia and would not elevate homocysteine. B_{12} deficiency is not most likely in this presentation.

10. **Answer: B.** Methylmalonyl-CoA mutase requires B_{12} but not folate for activity. Macrocytic anemia, elevated homocysteine, and macrocytic anemia can be caused by B_{12} or folate deficiency.

11. **Answer: A.** Progressive peripheral neuropathy. A distractor may be D, but this would be the cause of a B_{12} deficiency, not a result of it.

12. **Answer: B.** Acetyl-CoA activates pyruvate carboxylase and gluconeogenesis during fasting.

13. **Answer: F.** Fumarate.

14. **Answer: E.** Succinyl-CoA.

15. **Answer: D.** Glutamate is produced by B_6-dependent transamination of α-ketoglutarate.

16. **Answer: D.** Sideroblastic anemia in a person being treated for tuberculosis (with isoniazid) is most likely due to vitamin B_6 deficiency. δ-Aminolevulinate synthase, the first enzyme in heme synthesis, requires vitamin B_6 (pyridoxine).

17. **Answer: C.** Lead inhibits both ferrochelatase (increasing the zinc protoporphyrin) and ALA dehydrase (increasing δ-ALA).

18. **Answer: C.** The infant has a defect in the urea cycle, resulting from ornithine transcarbamylase (OTC) deficiency. OTC deficiency would result in decreased intermediates of the urea cycle, including decreased urea formation as indicated by the decreased BUN. OTC can be diagnosed by elevated orotic acid since carbamyl phosphate accumulates in the liver mitochondria and spills into the cytoplasm entering the pyrimidine-synthesis pathway.

    Methylmalonic acid in blood (**choice E**) is seen in vitamin B_{12} disorders.

    A decreased BUN would result in *elevated ammonia* in blood, raising the pH (**choices A and B**).

    Decreased BUN means decreased blood urea, hence, decreased urea in urine (**choice D**).

19. **Answer: B.** The above case describes a patient with Parkinson's disease, which is caused by degeneration of the substantia nigra. This leads to dopamine deficiency in the brain and results in resting tremors, bradykinesia, cog-wheeling of the hand joints, and rigidity of musculature. In addition, patients are often described as having "mask-like facies." Dopamine is one of the catecholamines synthesized in a common pathway with norepinephrine and epinephrine.

    The diseases involving amino acid degradation (**choice A**), ganglioside degradation (**choice C**), and sphingolipid degradation (**choice E**) do not match the presentation seen in the case.

Purine and Pyrimidine Metabolism 18

Learning Objectives

- ❑ Explain information related to pyrimidine synthesis
- ❑ Explain information related to purine synthesis
- ❑ Demonstrate understanding of purine catabolism and the salvage enzyme HGPRT

OVERVIEW

Nucleotides are needed for DNA and RNA synthesis (DNA replication and transcription) and for energy transfer. Nucleoside triphosphates (ATP and GTP) provide energy for reactions that would otherwise be extremely unfavorable in the cell.

Ribose 5-phosphate for nucleotide synthesis is derived from the hexose monophosphate shunt and is activated by the addition of pyrophosphate from ATP, forming phosphoribosyl pyrophosphate (PRPP) using PRPP synthetase. Cells synthesize nucleotides in 2 ways: *de novo* synthesis and salvage pathways.

- In ***de novo*** synthesis, which occurs predominantly in the liver, purines and pyrimidines are synthesized from smaller precursors, and PRPP is added to the pathway at some point.
- In the **salvage pathways**, preformed purine and pyrimidine bases can be converted into nucleotides by salvage enzymes distinct from those of *de novo* synthesis. Purine and pyrimidine bases for salvage enzymes may arise from:
  - Synthesis in the liver and transport to other tissues
  - Digestion of endogenous nucleic acids (cell death, RNA turnover)

In many cells, the capacity for *de novo* synthesis to supply purines and pyrimidines is insufficient, and the salvage pathway is essential for adequate nucleotide synthesis.

In Lesch-Nyhan disease, an enzyme for purine salvage (hypoxanthine guanine phosphoribosyl pyrophosphate transferase, HPRT) is absent or deficient. People with this genetic deficiency have CNS deterioration, mental retardation, and spastic cerebral palsy associated with compulsive self-mutilation. Cells in the basal ganglia of the brain (fine motor control) normally have very high HPRT activity. Patients also all have hyperuricemia because purines cannot be salvaged, causing gout.

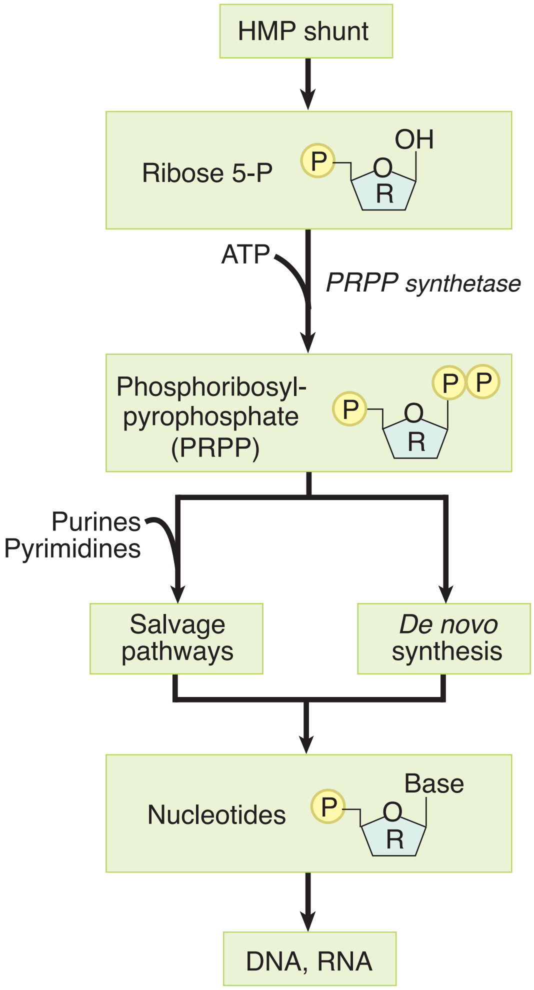


Figure I-18-1. Nucleotide Synthesis by Salvage and De Novo Pathways

PYRIMIDINE SYNTHESIS

Pyrimidines are synthesized *de novo* in the cytoplasm from aspartate, CO_2, and glutamine. Synthesis involves a cytoplasmic carbamoyl phosphate synthetase that differs from the mitochondrial enzyme with the same name used in the urea cycle.

Orotic Aciduria

> Several days after birth, an infant was observed to have severe anemia, which was found to be megaloblastic. There was no evidence of hepatomegaly or splenomegaly. The newborn was started on a bottle-fed regimen containing folate, vitamin B_{12}, vitamin B_6, and iron. One week later the infant's condition did not improve. The pediatrician noted that the infant's urine contained a crystalline residue, which was analyzed and determined to be orotic acid. Lab tests indicated no evidence of hyperammonemia. The infant was given a formula which contained uridine. Shortly thereafter, the infant's condition improved significantly.

Orotic aciduria is an autosomal recessive disorder caused by a defect in uridine monophosphate (UMP) synthase. This enzyme contains two activities, orotate phosphoribosyltransferase and orotidine decarboxylase. The lack of pyrimidines impairs nucleic acid synthesis needed for hematopoiesis, explaining the megaloblastic anemia in this infant. Orotic acid accumulates and spills into the urine, resulting in orotic acid crystals and orotic acid urinary obstruction. The presence of orotic acid in urine might suggest that the defect could be ornithine transcarbamylase (OTC) deficiency, but the lack of hyperammonemia rules out a defect in the urea cycle. Uridine administration relieves the symptoms by bypassing the defect in the pyrimidine pathway. Uridine is salvaged to UMP, which feedback-inhibits carbamoyl phosphate synthase-2, preventing orotic acid formation.

Note

Two Orotic Acidurias

- **Hyperammonemia** (no megaloblastic anemia)
  - Pathway: urea cycle
  - Enzyme deficient: OTC
- **Megaloblastic anemia** (no hyperammonemia)
  - Pathway: pyrimidine synthesis
  - Enzyme deficient: UMP synthase

Folate and B12 deficiency
(megaloblastic anemia but no orotic aciduria)

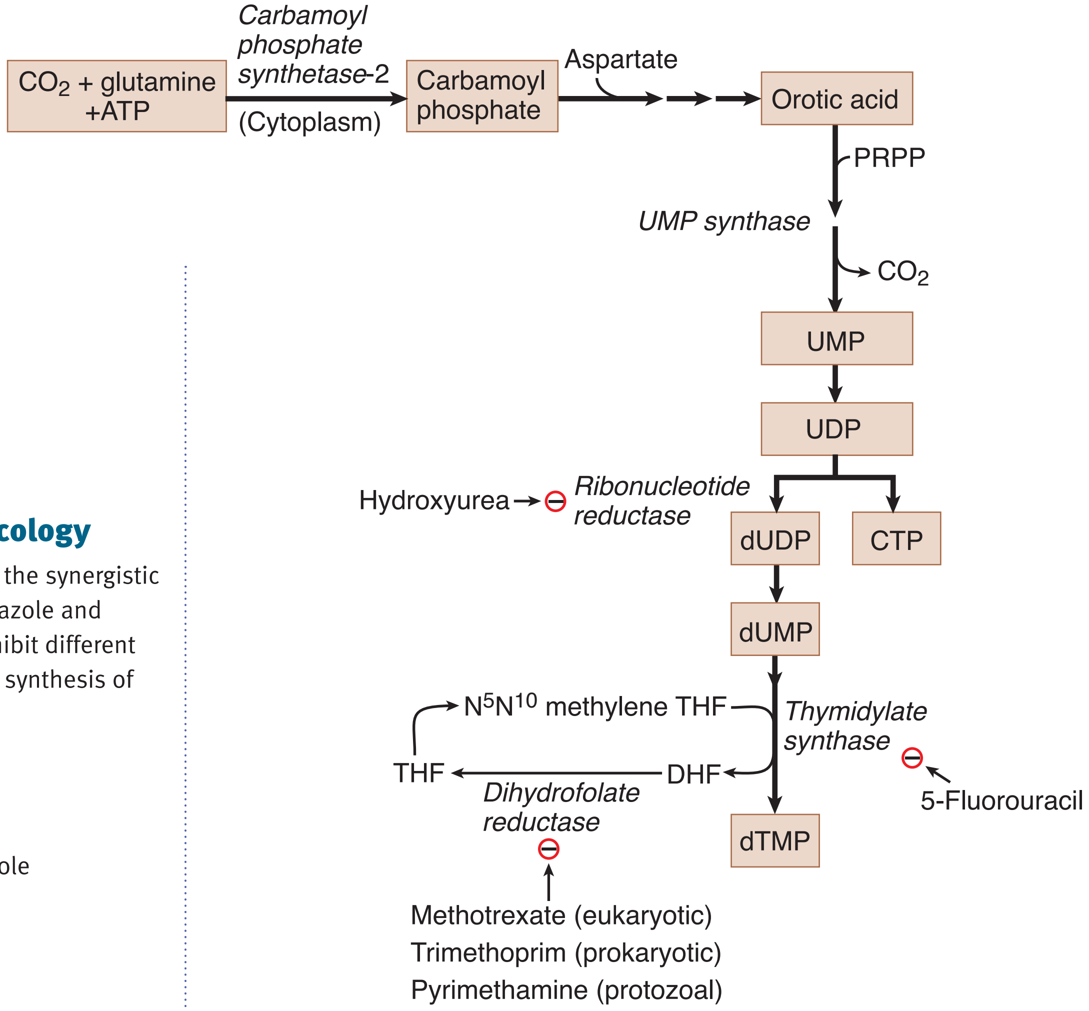


Figure I-18-2. *De Novo* Pyrimidine Synthesis

The primary end product of pyrimidine synthesis is UMP. In the conversion of UMP to dTMP, 3 important enzymes are ribonucleotide reductase, thymidylate synthase, and dihydrofolate reductase; all are targets of antineoplastic drugs.

Bridge to Pharmacology

Cotrimoxazole contains the synergistic antibiotics sulfamethoxazole and trimethoprim, which inhibit different steps in the prokaryotic synthesis of tetrahydrofolate.

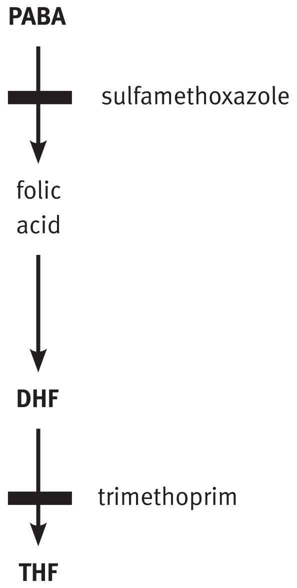


Table I-18-1. Important Enzymes of Pyrimidine Synthesis

Enzyme	Function	Drug
Ribonucleotide reductase	Reduces all NDPs to dNDPs for DNA synthesis	Hydroxyurea (S phase)
Thymidylate synthase	Methylates dUMP to dTMP Requires THF	5-Fluorouracil (S phase)
Dihydrofolate reductase (DHFR)	Converts DHF to THF Without DHFR, thymidylate synthesis will eventually stop	Methotrexate (eukaryotic) (S phase) Trimethoprim (prokaryotic) Pyrimethamine (protozoal)

Ribonucleotide Reductase

High-Yield

Ribonucleotide reductase is required for the formation of the deoxyribonucleotides for DNA synthesis.

- All 4 nucleotide substrates must be diphosphates.
- dADP and dATP strongly inhibit ribonucleotide reductase.
- Hydroxyurea, an anticancer drug, blocks DNA synthesis indirectly by inhibiting ribonucleotide reductase.

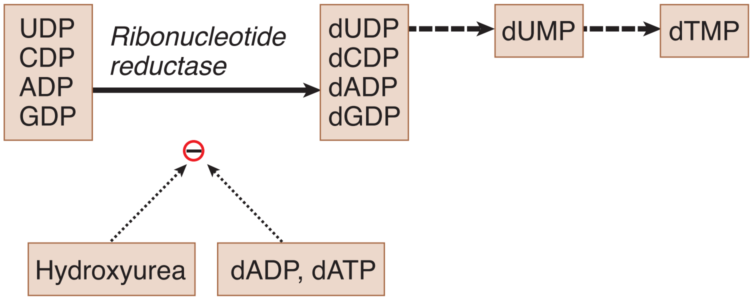


Figure I-18-3. Ribonucleotide Reductase

PYRIMIDINE CATABOLISM

Pyrimidines may be completely catabolized (NH_4^+ is produced) or recycled by pyrimidine salvage enzymes.

PURINE SYNTHESIS

Purines are synthesized *de novo* beginning with PRPP. The most important enzyme is PRPP amidotransferase, which catalyzes the first and rate-limiting reaction of the pathway. It is inhibited by the 3 purine nucleotide end products AMP, GMP, and IMP.

The drugs allopurinol (used for gout) and 6-mercaptopurine (antineoplastic) also inhibit PRPP amidotransferase. These drugs are purine analogs which must be converted to their respective nucleotides by HGPRT within cells.

- The amino acids glycine, aspartate, and glutamine are used in purine synthesis.
- Tetrahydrofolate is required for synthesis of all the purines.
- Inosine monophosphate (contains the purine base hypoxanthine) is the precursor for AMP and GMP.

Bridge to Microbiology

Protozoan and multicellular parasites and many obligate parasites, such as *Chlamydia*, cannot synthesize purines *de novo* because they lack the necessary genes in the purine pathway. However, they have elaborate salvage mechanisms for acquiring purines from the host to synthesize their own nucleic acids to grow.

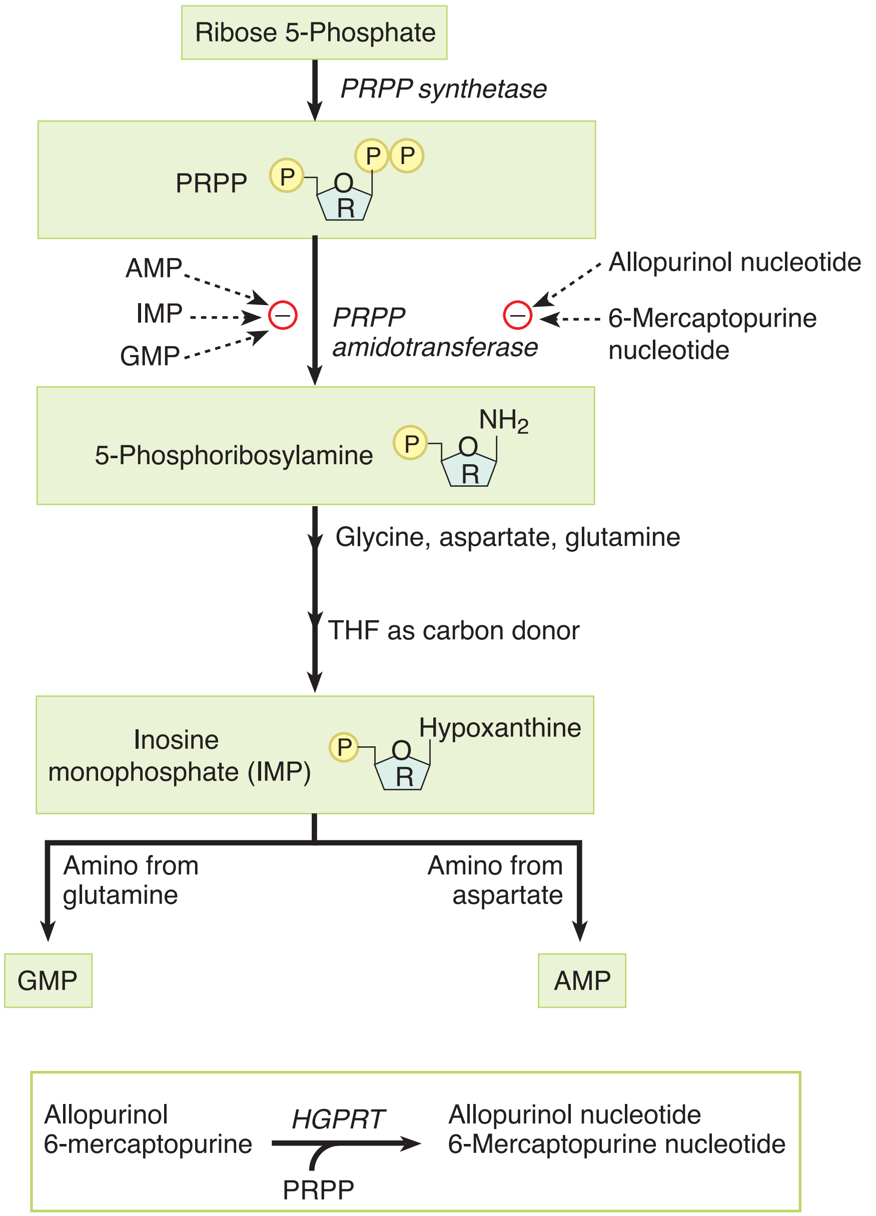


Figure I-18-4. *De Novo* Purine Synthesis

PURINE CATABOLISM AND THE SALVAGE ENZYME HGPRT

Excess purine nucleotides or those released from DNA and RNA by nucleases are catabolized first to nucleosides (loss of P_i) and then to free purine bases (release of ribose or deoxyribose). Excess nucleoside monophosphates may accumulate when:

- RNA is normally digested by nucleases (mRNAs and other types of RNAs are continuously turned over in normal cells).
- Dying cells release DNA and RNA, which is digested by nucleases.
- The concentration of free P_i decreases as it may in galactosemia, hereditary fructose intolerance, and glucose-6-phosphatase deficiency.

Salvage enzymes recycle normally about 90% of these purines, and 10% are converted to uric acid and excreted in urine. When purine catabolism is increased significantly, a person is at risk for developing hyperuricemia and potentially gout.

Purine catabolism to uric acid and salvage of the purine bases hypoxanthine (derived from adenosine) and guanine are shown below.

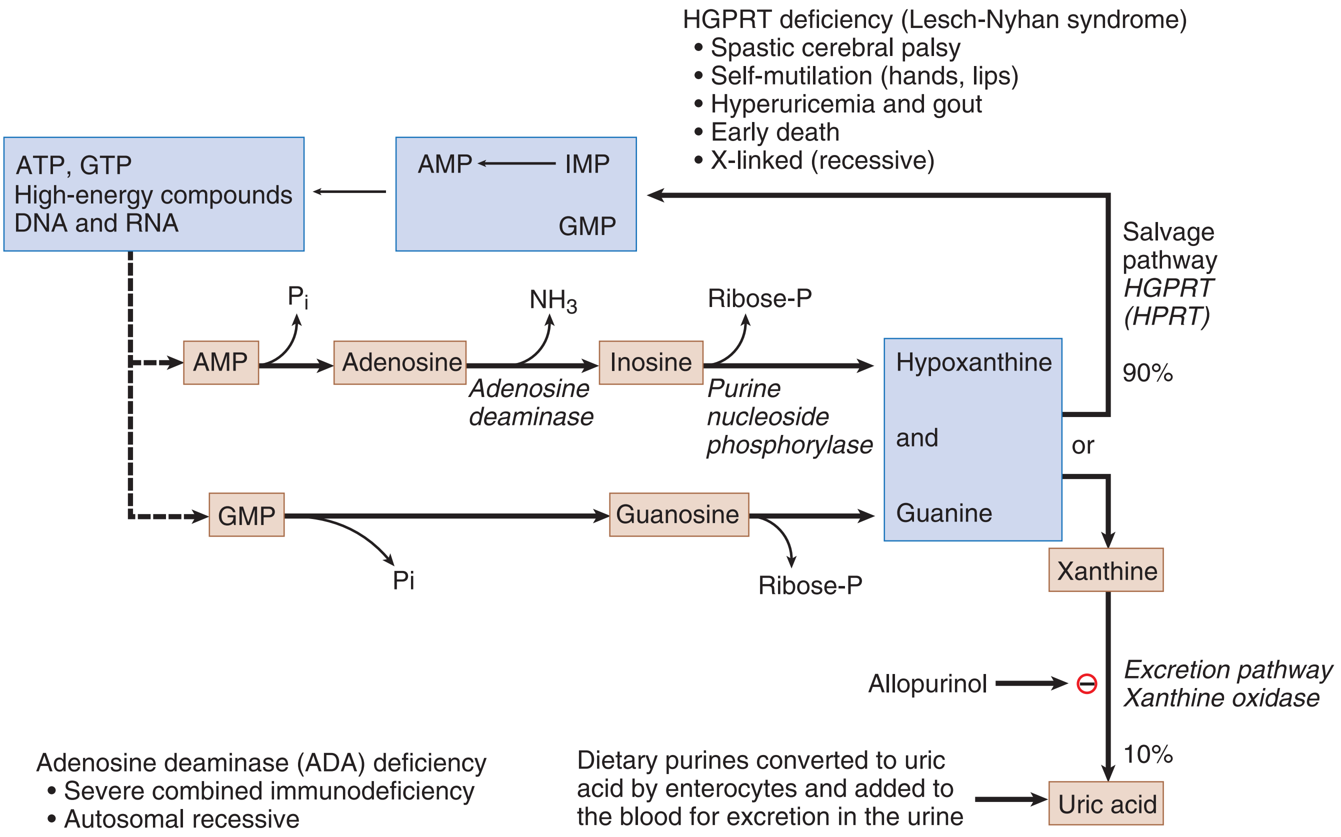


Figure I-18-5. Purine Excretion and Salvage Pathways

Adenosine Deaminase Deficiency

High-Yield

Adenosine deaminase (ADA) deficiency, an autosomal recessive disorder, causes a type of severe combined immunodeficiency (SCID). Lacking both B-cell and T-cell function, children are multiply infected with many organisms (*Pneumocystis carinii, Candida*) and do not survive without treatment. Enzyme replacement therapy and bone marrow transplantation may be used. Experimental gene therapy trials have not yet yielded completely successful cures.

High levels of dATP accumulate in red cells of ADA patients and inhibit ribonucleotide reductase, thereby inhibiting the production of other essential deoxynucleotide precursors for DNA synthesis (see Figure I-18-3). Although it is believed that the impaired DNA synthesis contributes to dysfunction of T cells and B cells, it is not known why the main effects are limited to these cell types.

Bridge to Pharmacology

Thiazide diuretics (hydrochlorothiazide and chlorthalidone) may cause hyperuricemia.

Hyperuricemia and Gout

High-Yield

Hyperuricemia may be produced by overproduction of uric acid or underexcretion of uric acid by the kidneys. Hyperuricemia may progress to acute and chronic gouty arthritis if uric acid (monosodium urate) is deposited in joints and surrounding soft tissue, where it causes inflammation. Uric acid is produced from excess endogenous purines as shown in Figure I-18-5, and is also produced from dietary purines (digestion of nucleic acid in the intestine) by intestinal epithelia. Both sources of uric acid are transported in the blood to the kidneys for excretion in urine.

Allopurinol inhibits xanthine oxidase and also can reduce purine synthesis by inhibiting PRPP amidotransferase, provided HGPRT is active (see Figure I-18-4). Hyperuricemia and gout often accompany the following conditions:

- Lesch-Nyhan syndrome (no purine salvage)
- Partial deficiency of HGPRT
- Alcoholism (lactate and urate compete for same transport system in the kidney)
- Glucose 6-phosphatase deficiency
- Hereditary fructose intolerance (aldolase B deficiency)
- Galactose 1-phosphate uridyl transferase deficiency (galactosemia)
- Mutations in PRPP synthetase that lower K_m

In the last 2 diseases, phosphorylated sugars accumulate, decreasing the available Pi and increasing AMP (which cannot be phosphorylated to ADP and ATP). The excess AMP is converted to uric acid.

Bridge to Pathology

Treatment of large tumors with chemotherapeutic regimens or radiation may cause "turnor lysis syndrome" and excessive excretion of uric acid, resulting in gout. The cause of the excessive uric acid is the destruction of the cancer cell's nucleic acid into purines undergoing turnover.

Clinical Correlate

Gout

Acute gouty arthritis, seen most commonly in males, results from precipitation of monosodium urate crystals in joints. The crystals, identified as negatively birefringent and needle-shaped, initiate neutrophil-mediated and acute inflammation, often first affecting the big toe. Chronic gout may manifest over time as tophi (deposits of monosodium urate) in soft tissue around joints, leading to chronic inflammation involving granulomas.

- Acute attacks of gout are treated with colchicine or indomethacin to reduce the inflammation.
- Chronic hyperuricemia, because of underexcretion, is treated with a uricosuric drug (probenecid).
- Overproduction of uric acid and chronic gout are treated with allopurinol.

Lesch-Nyhan Syndrome

High-Yield

Lesch-Nyhan syndrome is an X-linked recessive condition involving:

- Near-complete deficiency of HGPRT activity
- Mental retardation
- Spastic cerebral palsy with compulsive biting of hands and lips
- Hyperuricemia
- Death often in first decade

Over 100 distinct mutations of the HGPRT gene located on the X chromosome have been reported to give rise to Lesch-Nyhan syndrome. These mutations include complete deletions of the gene, point mutations that result in an increased K_m for hypoxanthine and guanine for the enzyme, and mutations that cause the encoded enzyme to have a short half-life.

Lesch-Nyhan Syndrome

The parents of a 9-month-old infant were concerned that their son appeared generally weak, had difficulty moving his arms and legs, repeatedly bit his lips, and frequently seemed to be in pain. The infant was brought to the pediatrician. The parents mentioned that since the baby was born, they often noticed tiny, orange-colored particles when they changed the infant's diapers. Lab analysis of uric acid in urine was normalized to the urinary creatinine in the infant, and it was found that the amount was 3 times greater than the normal range.

One of the earliest signs of Lesch-Nyhan syndrome is the appearance of orange crystals in diapers. They are needle-shaped sodium urate crystals. Without the salvaging of hypoxanthine and guanine by HGPRT, the purines are shunted toward the excretion pathway. This is compounded by the lack of regulatory control of the PRPP amidotransferase in the purine synthesis pathway, resulting in the synthesis of even more purines in the body. The large amounts of urate will cause crippling, gouty arthritis and urate nephropathy. Renal failure is usually the cause of death. Treatment with allopurinol will ease the amount of urate deposits formed.

Bridge to Pharmacology

Febuxostat is a nonpurine inhibitor of xanthine oxidase.

Bridge to Medical Genetics

There are a large number of known mutations in the HGPRT gene. These have varying effects on the K_m for the enzyme product, generating varying degrees of severity. This concept is known as allelic heterogeneity.

Review Questions

Select the ONE best answer.

1. A 6-month-old boy becomes progressively lethargic and pale and shows delayed motor development. Laboratory evaluation reveals normal blood urea nitrogen (BUN), low serum iron, hemoglobin 4.6 g/dL, and leukopenia. His bone marrow shows marked megaloblastosis, which did not respond to treatment with iron, folic acid, vitamin B_{12}, or pyridoxine. His urine developed abundant white precipitate identified as orotic acid. The underlying defect causing the megaloblastic anemia in this child is most likely in which of the following pathways?

   A. Homocysteine metabolism
   B. Pyrimidine synthesis
   C. Urea synthesis
   D. Uric acid synthesis
   E. Heme synthesis

2. Patients with Lesch-Nyhan syndrome have hyperuricemia, indicating an increased biosynthesis of purine nucleotides, and markedly decreased levels of hypoxanthine phosphoribosyl transferase (HPRT). The hyperuricemia can be explained on the basis of a decrease in which regulator of purine biosynthesis?

   A. ATP
   B. GDP
   C. Glutamine
   D. IMP
   E. PRPP

3. A 12-week-old infant with a history of persistent diarrhea and candidiasis is seen for a respiratory tract infection with *Pneumocystis jiroveci.* A chest x-ray confirms pneumonia and reveals absence of a thymic shadow. Trace IgG is present in his serum, but IgA and IgM are absent. His red blood cells completely lack an essential enzyme in purine degradation. The product normally formed by this enzyme is

   A. guanine monophosphate
   B. hypoxanthine
   C. inosine
   D. xanthine
   E. xanthine monophosphate

Items 4 and 5

The anticancer drug 6-mercaptopurine is deactivated by the enzyme xanthine oxidase. A cancer patient being treated with 6-mercaptopurine develops hyperuricemia, and the physician decides to give the patient allopurinol.

4. What effect will allopurinol have on the activity of 6-mercaptopurine?

   A. Enhanced deactivation of 6-mercaptopurine
   B. Enhanced elimination of 6-mercaptopurine as uric acid
   C. Enhanced retention and potentiation of activity
   D. Decreased inhibition of PRPP glutamylamidotransferase

5. Resistance of neoplastic cells to the chemotherapeutic effect of 6-mercaptopurine would most likely involve loss or inactivation of a gene encoding

   A. thymidylate synthase
   B. hypoxanthine phosphoribosyltransferase
   C. purine nucleoside pyrophosphorylase
   D. orotic acid phosphoribosyltransferase
   E. adenosine deaminase

Answers

1. **Answer: B.** Accumulation of orotic acid indicates megaloblastic anemia arises because pyrimidines are required for DNA synthesis.

2. **Answer: D.** IMP is a feedback inhibitor of PRPP amidophosphoribosyl transferase, the first reaction in the biosynthesis of purines. IMP is formed by the HPRT reaction in the salvage of hypoxanthine.

3. **Answer: C.** The child most likely has severe combined immunodeficiency caused by adenosine deaminase deficiency. This enzyme deaminates adenosine (a nucleoside) to form inosine (another nucleoside). Hypoxanthine and xanthine are both purine bases, and the monophosphates are nucleotides.

4. **Answer: C.** Because allopurinol inhibits xanthine oxidase, the 6-mercaptopurine will not be deactivated as rapidly.

5. **Answer: B.** HPRT is required for activation of 6-mercaptopurine to its ribonucleotide and inhibition of purine synthesis. The other enzymes listed are not targets for this drug.

PART II

Medical Genetics

Single-Gene Disorders 1

Learning Objectives

- ❑ Interpret scenarios about basic definitions
- ❑ Use knowledge of major modes of inheritance
- ❑ Understand important principles that can characterize single-gene diseases

BASIC DEFINITIONS

Chromosomes

Humans are composed of 2 groups of cells:

- **Gametes.** Ova and sperm cells, which are haploid, have one copy of each type of chromosome (1–22, X or Y). This DNA is transmitted to offspring.
- **Somatic cells** (cells other than gametes). Nearly all somatic cells are diploid, having 2 copies of each type of autosome (1–22) and either XX or XY.

Diploid cells

- **Homologous chromosomes.** The 2 chromosomes in each diploid pair are said to be homologs, or homologous chromosomes. They contain the same genes, but because one is of paternal origin and one is of maternal origin, they may have different alleles at some loci.
- **X and Y chromosomes,** or the sex chromosomes, have some homologous regions but the majority of genes are different. The regions that are homologous are sometimes referred to as pseudoautosomal regions. During meiosis-1 of male spermatogenesis, the X and Y chromosomes pair in the pseudoautosomal regions, allowing the chromosomes to segregate into different cells.

Genes

- **Gene.** Physically a gene consists of a sequence of DNA that encodes a specific protein (or a nontranslated RNA; for example: tRNA, rRNA, or snRNA).
- **Locus.** The physical location of a gene on a chromosome is termed a locus.
- **Alleles.** Variation (mutation) in the DNA sequence of a gene produces a new allele at that locus. Many genes have multiple alleles.
- **Polymorphism.** When a specific site on a chromosome has multiple alleles in the population, it is said to be polymorphic (many forms).

Note

- **Gene:** basic unit of inheritance
- **Locus**: location of a gene on a chromosome
- **Allele**: different forms of a gene
- **Genotype**: alleles found at a locus
- **Phenotype**: physically observable features
- **Homozygote**: alleles at a locus are the same
- **Heterozygote**: alleles at a locus are different
- **Dominant**: requires only one copy of the mutation to produce disease
- **Recessive**: requires 2 copies of the mutation to produce disease

Note

Although the term *alleles* is used most frequently with genes, noncoding DNA can also have alleles of specific sequences.

For example, the β-globin gene encodes a protein (β-globin). It has been mapped to chromosome 11p15.5 indicating its locus, a specific location on chromosome 11. Throughout human history there have been many mutations in the β-globin gene, and each mutation has created a new allele in the population. The β-globin locus is therefore polymorphic. Some alleles cause no clinical disease, but others, like the sickle cell allele, are associated with significant disease. Included among the disease-causing alleles are those associated with sickle cell anemia and several associated with β-thalassemia.

Genotype

The specific DNA sequence at a locus is termed a genotype. In diploid somatic cells a genotype may be:

- **Homozygous** if the individual has the same allele on both homologs (homologous chromosomes) at that locus.
- **Heterozygous** if the individual has different alleles on the two homologs (homologous chromosomes) at that locus.

Note

Major types of single-gene mutations are:

- Missense
- Nonsense
- Deletion
- Insertion
- Frameshift

Phenotype

The phenotype is generally understood as the expression of the genotype in terms of observable characteristics.

Mutations

A mutation is an alteration in DNA sequence (thus, mutations produce new alleles). When mutations occur in cells giving rise to gametes, the mutations can be transmitted to future generations. *Missense* mutations result in the substitution of a single amino acid in the polypeptide chain (e.g., sickle cell disease is caused by a missense mutation that produces a substitution of valine for glutamic acid in the β-globin polypeptide). *Nonsense* mutations produce a stop codon, resulting in premature termination of translation and a truncated protein. Nucleotide bases may be inserted or deleted. When the number of inserted or deleted bases is a multiple of 3, the mutation is said to be *in-frame***.** If not a multiple of 3, the mutation is a *frameshift,* which alters all codons downstream of the mutation, typically producing a truncated or severely altered protein product. Mutations can occur in promoter and other regulatory regions or in genes for transcription factors that bind to these regions. This can decrease or increase the amount of gene product produced in the cell. (For a complete description of these and other mutations, see Section I, Chapter 4: Translation; Mutations.)

Mutations can also be classified according to their phenotypic effects. Mutations that cause a missing protein product or cause decreased activity of the protein are termed *loss-of-function*. Those that produce a protein product with a new function or increased activity are termed *gain-of-function*.

Recurrence risk

The *recurrence risk* is the probability that the offspring of a couple will express a genetic disease. For example, in the mating of a normal homozygote with a heterozygote who has a dominant disease-causing allele, the recurrence risk for each offspring is 1/2, or 50%. It is important to remember that each reproductive event is statistically independent of all previous events. Therefore, the recurrence risk remains the same regardless of the number of previously affected or unaffected offspring. Determining the mode of inheritance of a disease

(e.g., autosomal dominant versus autosomal recessive) enables one to assign an appropriate recurrence risk for a family.

Pedigrees

A patient's family history is diagrammed in a pedigree. The first affected individual to be identified in the family is termed the **proband**.

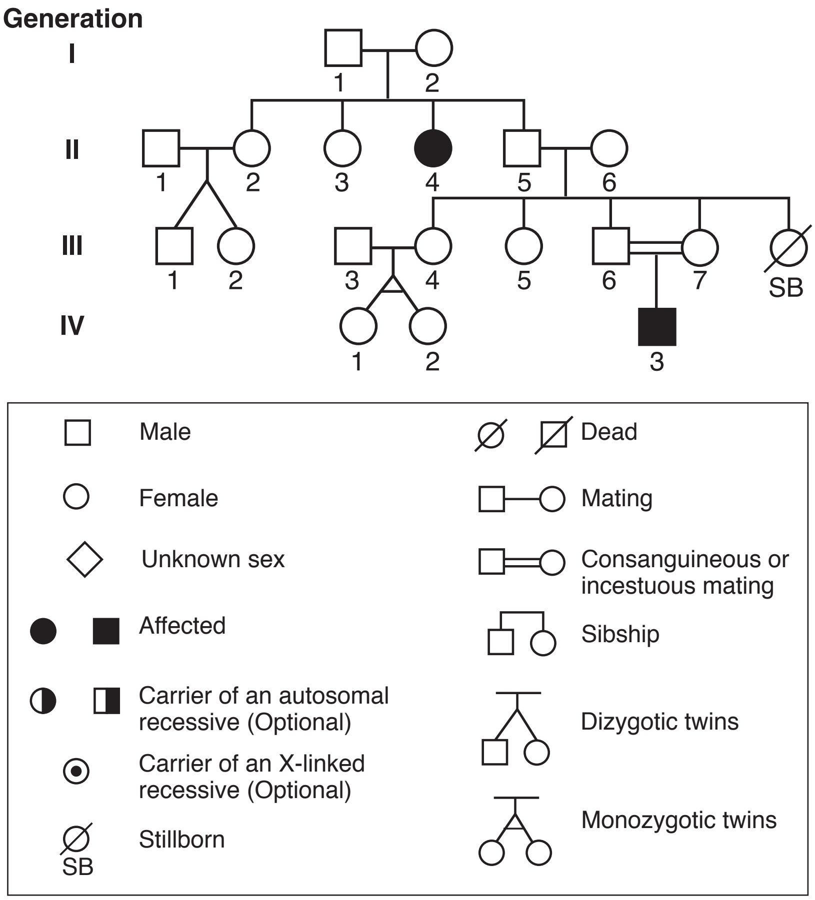


Figure II-1-1. Pedigree Nomenclature

MAJOR MODES OF INHERITANCE

Autosomal Dominant Inheritance

High-Yield

A number of features in a pedigree help identify autosomal dominant inheritance:

- Because affected individuals must receive a disease-causing gene from an affected parent, the disease is typically observed in multiple generations of a pedigree.
- Skipped generations are not typically seen because two unaffected parents cannot transmit a disease-causing allele to their offspring (an exception occurs when there is reduced penetrance).
- Because these genes are located on autosomes, males and females are affected in roughly equal frequencies.

Autosomal dominant alleles are relatively rare in populations, so the typical mating pattern is a heterozygous affected individual (Aa genotype) mating with a homozygous normal individual (aa genotype). Note that, by convention, the dominant allele is shown in uppercase (A) and the recessive allele is shown in lowercase (a). The recurrence risk is thus 50%, and half the children, on average, will be affected with the disease. If both parents are heterozygous, the recurrence risk is 75%.

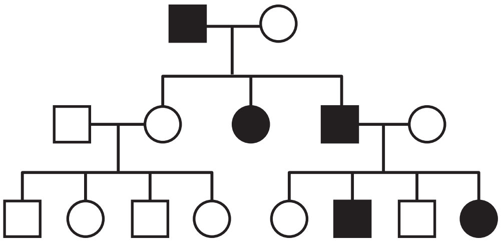


Figure II-1-2. Autosomal Dominant Inheritance

	A	a
a	Aa	aa
a	Aa	aa

A Punnett square: Affected offspring (Aa) are shaded.

Figure II-1-3. Recurrence Risk for the Mating of Affected Individual (Aa) with a Homozygous Unaffected Individual (aa) using a Punnett Square

Autosomal Recessive Inheritance

High-Yield

Important features that distinguish autosomal recessive inheritance:

- Because autosomal recessive alleles are clinically expressed only in the homozygous state, the offspring must inherit one copy of the disease-causing allele from each parent.
- In contrast to autosomal dominant diseases, autosomal recessive diseases are typically seen in only one generation of a pedigree.
- Because these genes are located on autosomes, males and females are affected in roughly equal frequencies.

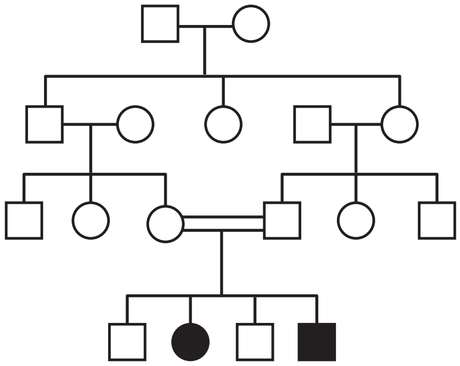
Most commonly, a homozygote is produced by the union of two heterozygous (*carrier*) parents. The recurrence risk for offspring of such matings is 25%.

Note

Autosomal Dominant Diseases

- Familial hypercholesterolemia (LDL receptor deficiency)
- Huntington disease
- Neurofibromatosis type 1
- Marfan syndrome
- Acute intermittent porphyria

Consanguinity (the mating of related individuals) is sometimes seen in recessive pedigrees because individuals who share common ancestors are more likely to carry the same recessive disease-causing alleles.



A consanguineous mating has produced two affected offspring.

Figure II-1-4. Pedigree for an Autosomal Recessive Disease

Determining the Recurrence Risk for an Individual Whose Phenotype Is Known. In Figure II-1-4, individual IV-1 may wish to know his risk of being a carrier. Because his phenotype is known, there are only 3 possible genotypes he can have, assuming complete penetrance of the disease-producing allele. He cannot be homozygous for the recessive allele (aa). Two of the remaining 3 possibilities are carriers (Aa and aA), and one is homozygous normal (AA). Thus, his risk of being a carrier is 2/3, or 0.67 (67%).

	A	a
A	AA	Aa
a	Aa	aa

The affected genotype (aa) is shaded.

Figure II-1-5. Recurrence Risk for the Mating of Two Heterozygous Carriers (Aa) of a Recessive Mutation

X-Linked Recessive Inheritance

High-Yield

Properties of X-linked recessive inheritance

Because males have only one copy of the X chromosome, they are said to be **hemizygous** (*hemi* = "half") for the X chromosome. If a recessive disease-causing mutation occurs on the X chromosome, a male will be affected with the disease.

- Because males require only one copy of the mutation to express the disease and females require 2 copies, X-linked recessive diseases are seen much more commonly in males than in females.

Note

Autosomal Recessive Diseases

- Sickle cell anemia
- Cystic fibrosis
- Phenylketonuria (PKU)
- Tay-Sachs disease (hexosaminidase A deficiency)

Note

Cystic fibrosis is an important topic on the exam.

- Skipped generations are commonly seen because an affected male can transmit the disease-causing mutation to a heterozygous daughter, who is unaffected but who can transmit the disease-causing allele to her sons.
- Male-to-male transmission is not seen in X-linked inheritance; this helps distinguish it from autosomal inheritance.

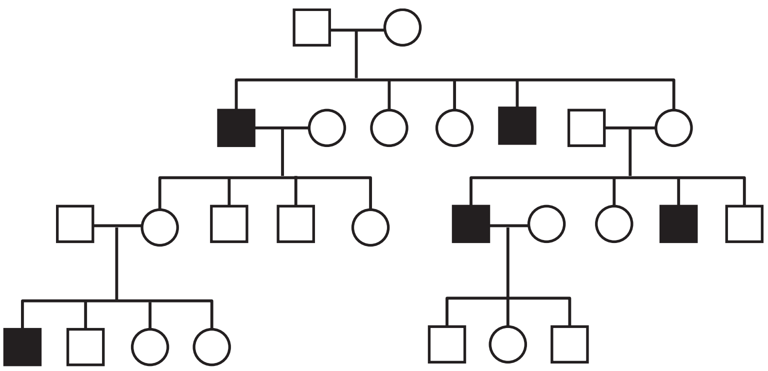


Figure II-1-6. X-Linked Recessive Inheritance

Note

X-Linked Recessive Diseases

- Duchenne muscular dystrophy
- Lesch-Nyhan syndrome (hypoxanthine-guanine phosphoribosyltransferase [HGPRT] deficiency)
- Glucose-6-phosphate dehydrogenase deficiency
- Hemophilia A and B
- Red-green color blindness
- Menke’s disease
- Ornithine transcarbamoylase (OTC) deficiency
- SCID (IL-receptor γ-chain deficiency)

Recurrence risks

Figure II-1-7 shows the recurrence risks for X-linked recessive diseases.

- Affected male–homozygous normal female: All of the daughters will be heterozygous carriers; all of the sons will be homozygous normal.
- Normal male–carrier female: On average, half of the sons will be affected and half of the daughters will be carriers. Note that in this case, the recurrence rate is different depending on the sex of the child. If the fetal sex is known, the recurrence rate for a daughter is 0, and that for a son is 50%. **If the sex of the fetus is not known, then the recurrence rate is multiplied by 1/2, the probability that the fetus is a male versus a female. Therefore if the sex is unknown, the recurrence risk is 25%.**

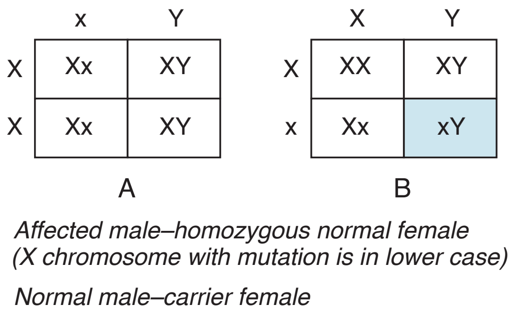


Figure II-1-7. Recurrence Risks for X-Linked Recessive Diseases

X inactivation

Normal males inherit an X chromosome from their mother and a Y chromosome from their father, whereas normal females inherit an X chromosome from each parent. Because the Y chromosome carries only about 50 protein-coding genes and the X chromosome carries hundreds of protein-coding genes, a mechanism must exist to equalize the amount of protein encoded by X chromosomes in males and females. This mechanism, termed X inactivation, occurs in the blastocyst (~100 cells) during the development of female embryos. When an X chromosome is inactivated, its DNA is not transcribed into mRNA, and the chromosome is visualized under the microscope as a highly condensed *Barr body* in the nuclei of interphase cells. X inactivation has several important characteristics:

- It is *random*—in some cells of the female embryo, the X chromosome inherited from the father is inactivated, and in others the X chromosome inherited from the mother is inactivated. Like coin tossing, this is a random process. As shown in Figure II-1-6, most women have their paternal X chromosome active in approximately 50% of their cells and the maternal X chromosome active in approximately 50% of their cells. Thus, females are said to be *mosaics* with respect to the active X chromosome.
- It is *fixed*—once inactivation of an X chromosome occurs in a cell, the same X chromosome is inactivated in all descendants of the cell.
- It is *incomplete*—there are regions throughout the X chromosome, including the tips of both the long and short arms, that are not inactivated.
- X-chromosome inactivation is permanent in somatic cells and reversible in developing germ line cells. Both X chromosomes are active during oogenesis.
- All X chromosomes in a cell are inactivated except one. For example, females with 3 X chromosomes in each cell (see Chapter 3) have two X chromosomes inactivated in each cell (thus, two Barr bodies can be visualized in an interphase cell). **X-chromosome inactivation is thought to be mediated by >1 mechanism.**
- A gene called *XIST* has been identified as the primary gene that causes X inactivation. *XIST* produces an RNA product that coats the chromosome, helping produce its inactivation.
- Condensation into heterochromatin
- Methylation of gene regions on the X chromosome

Note

X inactivation occurs early in the female embryo and is random, fixed, and incomplete. In a cell, all X chromosomes but one are inactivated.

Note

Genetic mosaicism is the presence of 2 or more cell lines with different karyotypes in an individual. It arises from mitotic nondisjunction. The number of cell lines that develop and their relative proportions are influenced by the *timing* of nondisjunction during embryogenesis and the *viability* of the aneuploid cells produced.

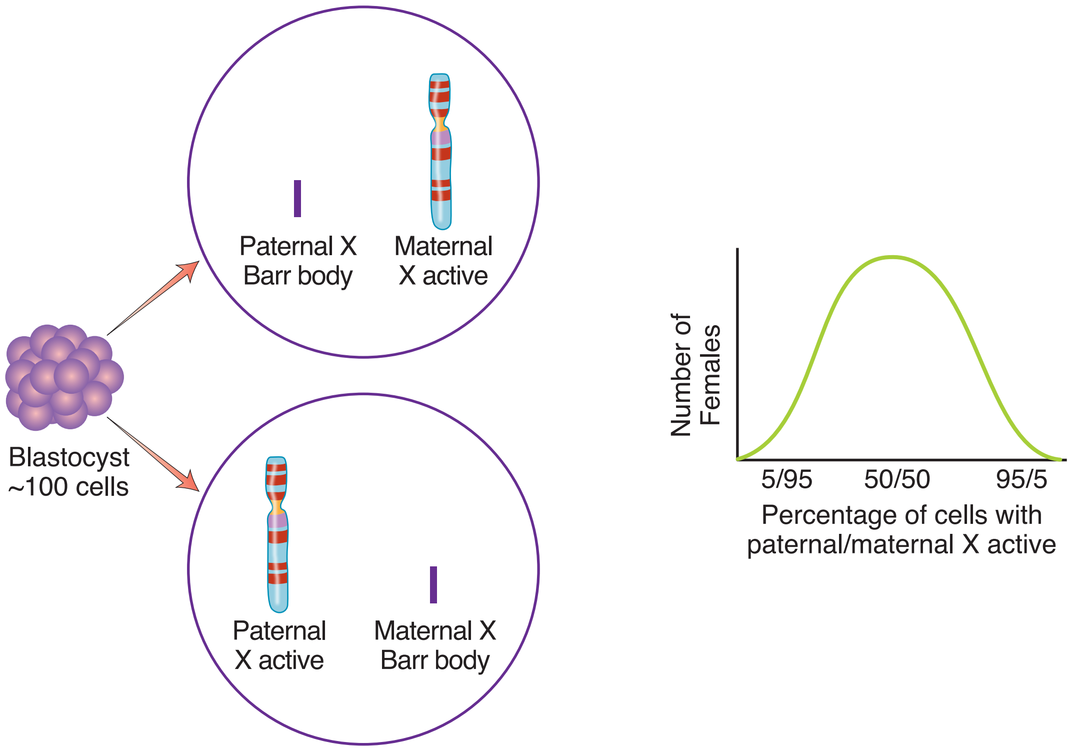


Figure II-1-8. Inactivation of the X Chromosome during Embryogenesis Is a Random Process

Manifesting (female) heterozygotes

Normal females have two copies of the X chromosome, so they usually require two copies of the mutation to express the disease. However, because X inactivation is a random process, a heterozygous female will occasionally express an X-linked recessive mutation because, by random chance, most of the X chromosomes carrying the normal allele have been inactivated. Such females are termed *manifesting heterozygotes*. Because they usually have at least a small population of active X chromosomes carrying the normal allele, their disease expression is typically milder than that of hemizygous males.

Recall Question

Which of the following is an X-linked recessive disease?

A. Acute intermittent porphyria

B. Duchenne muscular dystrophy

C. Huntington disease

D. Marfan syndrome

Answer: B

Y Chromosome Highlights

- The *SRY* (sex determining region) gene is a transcription factor that initiates male development.
- The q arm of Y chromosomes contains a large block of heterochromatin.
- Microdeletions of Yq in males result in nonobstructive azoospermia.

X-Linked Dominant Inheritance

High-Yield

There are relatively few diseases whose inheritance is classified as X-linked dominant. Fragile X syndrome is an important example. In this condition, females are differently affected than males, and whereas penetrance in males is 100%, that in females is approximately 60%. The typical fragile X patient described is male.

As in X-linked recessive inheritance, male–male transmission of the disease-causing mutation is not seen.

- Heterozygous females are affected. Because females have 2 X chromosomes (and thus 2 chances to inherit an X-linked disease-causing mutation) and males have only one, X-linked dominant diseases are seen about twice as often in females as in males.
- As in autosomal dominant inheritance, the disease phenotype is seen in multiple generations of a pedigree; skipped generations are relatively unusual.
- Examine the children of an affected male (II-1 in the figure below). None of his sons will be affected, but all of his daughters have the disease (assuming complete penetrance).

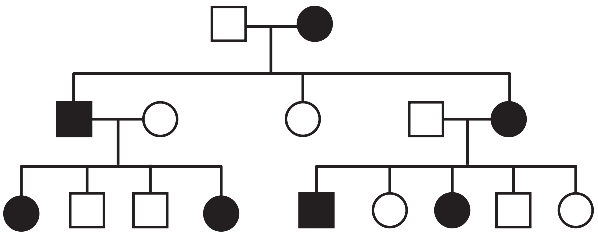


Figure II-1-9. X-Linked Dominant Inheritance

Recurrence Risks

Figure II-1-10 shows the recurrence risks for X-linked dominant inheritance.

- Affected male–homozygous normal female: None of the sons are affected; all of the daughters are affected. Note that in this case, the recurrence rate is different depending on the sex of the child. If the fetal sex is known, the recurrence rate for a daughter is 100%, and that for a son is 0%. **If the sex of the fetus is not known, then the recurrence rate is multiplied by 1/2, the probability that the fetus is a male versus a female. Therefore if the sex is unknown, the recurrence risk is 50%.**
- Normal male–heterozygous affected female: on average, 50% of sons are affected and 50% of daughters are affected.

Clinical Correlate

Fragile X Syndrome

Males: 100% penetrance

- Mental retardation
- Large ears
- Prominent jaw
- Macro-orchidism (usually postpubertal)

Females: 60% penetrance

- Mental retardation

Note

The **penetrance** of a disease-causing mutation is the percentage of individuals who are known to have the disease-causing genotype who display the disease phenotype (develop symptoms).

Note

X-Linked Dominant Diseases

- Hypophosphatemic rickets
- Fragile X syndrome

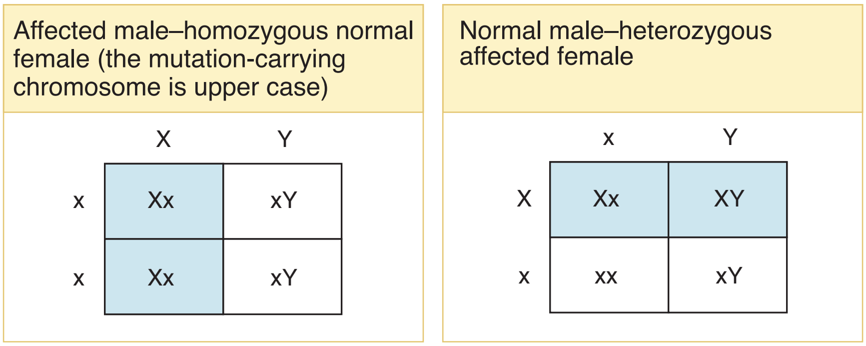
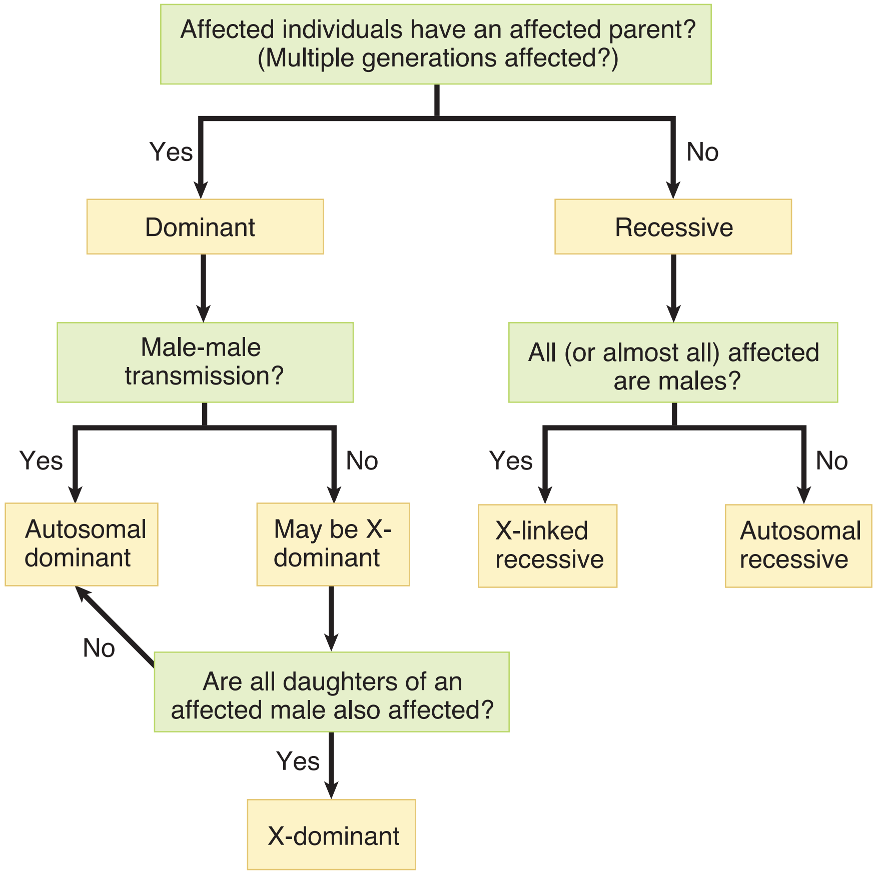


Figure II-1-10. Recurrence Risks for X-Linked Dominant Inheritance



Note: If transmission occurs *only* through affected mothers and never through affected sons, the pedigree is likely to reflect mitochondrial inheritance.

Figure II-1-11. A Basic Decision Tree for Determining the Mode of Inheritance in a Pedigree

Mitochondrial Inheritance

High-Yield

Mitochondria, which are cytoplasmic organelles involved in cellular respiration, have their own chromosomes, each of which contains 16,569 DNA base pairs (bp) arranged in a circular molecule. This DNA encodes 13 proteins that are subunits of complexes in the electron transport and oxidative phosphorylation processes (see Part I, Chapter 13). In addition, mitochondrial DNA encodes 22 transfer RNAs and 2 ribosomal RNAs.

Because a sperm cell contributes no mitochondria to the egg cell during fertilization, mitochondrial DNA is inherited exclusively through females. Pedigrees for mitochondrial diseases thus display a distinct mode of inheritance: Diseases are transmitted only from affected females to their offspring.

- Both males and females are affected.
- Transmission of the disease is only from a female.
- All offspring of an affected female are affected.
- None of the offspring of an affected male is affected.
- Diseases are typically neuropathies and/or myopathies.

Heteroplasmy

A typical cell contains hundreds of mitochondria in its cytoplasm, and each mitochondrion has its own copy of the mitochondrial genome. When a specific mutation occurs in some of the mitochondria, this mutation can be unevenly distributed into daughter cells during cell division: Some cells may inherit more mitochondria in which the normal DNA sequence predominates, while others inherit mostly mitochondria with the mutated, disease-causing gene. This condition is known as *heteroplasmy*. Variations in heteroplasmy account for substantial variation in the severity of expression of mitochondrial diseases.

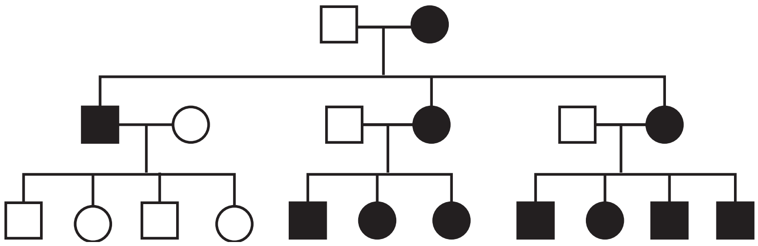


Figure II-1-12. Pedigree for a Mitochondrial Disease

Note

Mitochondrial Diseases

- Leber hereditary optic neuropathy
- MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
- Myoclonic epilepsy with ragged red muscle fibers

IMPORTANT PRINCIPLES THAT CAN CHARACTERIZE SINGLE-GENE DISEASES

Variable Expression

Hemochromatosis

Mary B. is a 45-year-old white female with hip pain of 2 years' duration. She also experiences moderate chronic fatigue. Routine blood work shows that liver function tests (LFTs) are slightly elevated. She does not drink alcohol. She takes no prescription drugs although she does use aspirin for the hip pain. She takes no vitamin or mineral supplements.

Mary B.'s 48-year-old brother has recently been diagnosed with hereditary hemochromatosis. Her brother's symptoms include arthritis for which he takes Tylenol (acetaminophen), significant hepatomegaly, diabetes, and "bronze" skin. His transferrin saturation is 75% and ferritin 1300 ng/mL. A liver biopsy revealed stainable iron in all hepatocytes and initial indications of hepatic cirrhosis. He was found to be homozygous for the most common mutation (C282Y) causing hemochromatosis. Subsequently Mary was tested and also proved to be homozygous for the C282Y mutation. Following diagnosis, both individuals were treated with periodic phlebotomy to satisfactorily reduce iron load.

Most genetic diseases vary in the degree of phenotypic expression: Some individuals may be severely affected, whereas others are more mildly affected. This can be the result of several factors.

Environmental Influences. In the case of hemochromatosis described above, Mary's less-severe phenotype may in part be attributable to loss of blood during regular menses throughout adulthood. Her brother's use of Tylenol may contribute to his liver problems.

The autosomal recessive disease xeroderma pigmentosum will be expressed more severely in individuals who are exposed more frequently to ultraviolet radiation.

Allelic Heterogeneity. Different mutations in the disease-causing locus may cause more- or less-severe expression. Most genetic diseases show some degree of allelic heterogeneity. For example, missense mutations in the factor VIII gene tend to produce less severe hemophilia than do nonsense mutations, which result in a truncated protein product and little, if any, expression of factor VIII.

Allelic heterogeneity usually results in phenotypic variation between families, not within a single family. Generally the same mutation is responsible for all cases of the disease within a family. In the example of hemochromatosis above, both Mary and her brother have inherited the same mutation; thus, allelic heterogeneity is not responsible for the variable expression in this case.

It is relatively uncommon to see a genetic disease in which there is no allelic heterogeneity.

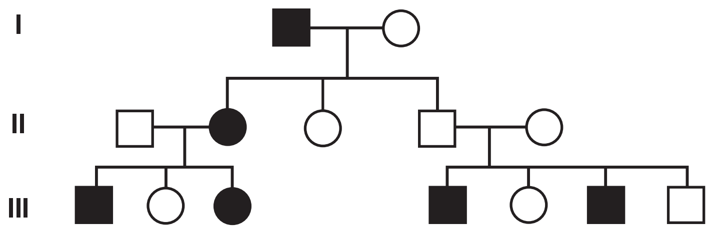
Heteroplasmy in mitochondrial pedigrees.

Modifier Loci. Disease expression may be affected by the action of other loci, termed modifier loci. Often these may not be identified.

Incomplete Penetrance

High-Yield

A disease-causing mutation is said to have incomplete penetrance when some individuals who have the disease genotype (e.g., one copy of the mutation for an autosomal dominant disease or two copies for an autosomal recessive disease) do not display the disease phenotype. Incomplete penetrance is distinguished from variable expression in that the nonpenetrant gene has no phenotypic expression at all. In the pedigree shown below, individual II-4 must have the disease-causing allele (he passed it from his father to his son) but shows no symptoms. He is an example of nonpenetrance.



The unaffected male in generation II (II-4) has an affected father and two affected sons. He must have the disease-causing mutation, although it shows incomplete penetrance.

Figure II-1-13. Incomplete Penetrance for an Autosomal Dominant Disease

The penetrance of a disease-causing mutation is quantified by examining a large number of families and calculating the percentage of individuals who are known to have the disease-causing genotype who display the disease phenotype.

Suppose that we had data from several different family studies of the disease affecting the family above and had identified 50 individuals with the disease-producing genotype. Of these individuals only 40 had any symptom(s). Penetrance would be calculated as:

$$40/50 = 0.80, \text{ or } 80\%$$

Penetrance must be taken into account when predicting recurrence risks. For instance, if II-1 and II-2 have another child, the recurrence risk is:

$$0.50 \times 0.80 = 0.40, \text{ or } 40\%$$

Both dominant diseases and recessive diseases can show incomplete (reduced) penetrance.

- Although 1 in 300 whites inherits the homozygous genotype for hemochromatosis, a much smaller percentage of individuals develop the disease (approximately 1 in 1,000–2,000). Penetrance for this autosomal recessive disease is only about 15%.

Notice that hereditary hemochromatosis is an example of incomplete penetrance and also an example of variable expression. Expression of the disease phenotype in individuals homozygous for the disease-causing mutation can run the gamut from severe symptoms to none at all. Among the 15% of individuals with at least some phenotypic expression, that expression can be more or less

severe (variable expression). However, 85% of individuals homozygous for the disease-causing mutation never have any symptoms (nonpenetrance). The same factors that contribute to variable expression in hemochromatosis can also contribute to incomplete penetrance.

It is necessary to be able to:

- Define incomplete (reduced) penetrance.
- Identify an example of incomplete penetrance in an autosomal dominant pedigree as shown in Figure II-1-13.
- Include penetrance in a simple recurrence risk calculation.

Incomplete Penetrance in Familial Cancer. Retinoblastoma is an autosomal dominant condition caused by an inherited loss-of-function mutation in the *Rb* tumor suppressor gene. In 10% of individuals who inherit this mutation, there is no additional somatic mutation in the normal copy and retinoblastoma does not develop, although they can pass the mutation to their offspring. Penetrance of retinoblastoma is therefore 90%.

Pleiotropy

Pleiotropy exists when a single disease-causing mutation affects multiple organ systems. Pleiotropy is a common feature of genetic diseases.

Pleiotropy in Marfan Syndrome

Marfan syndrome is an autosomal dominant disease that affects approximately 1 in 10,000 individuals. It is characterized by skeletal abnormalities (thin, elongated limbs; pectus excavatum; pectus carinatum), hypermobile joints, ocular abnormalities (frequent myopia and detached lens), and most importantly, cardiovascular disease (mitral valve prolapse and aortic aneurysm). Dilatation of the ascending aorta is seen in 90% of patients and frequently leads to aortic rupture or congestive heart failure. Although the features of this disease seem rather disparate, they are all caused by a mutation in the gene that encodes fibrillin, a key component of connective tissue. Fibrillin is expressed in the periosteum and perichondrium, the suspensory ligament of the eye, and the aorta. Defective fibrillin causes the connective tissue to be “stretchy” and leads to all of the observed disease features. Marfan syndrome thus provides a good example of the principle of pleiotropy.

Locus Heterogeneity

Locus heterogeneity exists when the same disease phenotype can be caused by mutations in different loci. Locus heterogeneity becomes especially important when genetic testing is performed by testing for mutations at specific loci.

Locus Heterogeneity in Osteogenesis Imperfecta Type 2

Osteogenesis imperfecta (OI) is a disease of bone development that affects approximately 1 in 10,000 individuals. It results from a defect in the collagen protein, a major component of the bone matrix. Four types of OI have been identified.

Type 2, the severe perinatal type, is the result of a defect in type 1 collagen, a trimeric molecule that has a triple helix structure. Two members of the trimer are encoded by a gene on chromosome 17, and the third is encoded by a gene on chromosome 7. Mutations in either of these genes give rise to a faulty collagen molecule, causing type 2 OI. Often, patients with chromosome 17 mutations are clinically indistinguishable from those with chromosome 7 mutations. This exemplifies the principle of locus heterogeneity.

New Mutations

In many genetic diseases, particularly those in which the mortality rate is high or the fertility rate is low, a large proportion of cases are caused by a new mutation transmitted from an unaffected parent to an affected offspring. There is thus no family history of the disease (for example, 100% of individuals with osteogenesis imperfecta type 2, discussed above, are the result of a new mutation in the family). A pedigree in which there has been a new mutation is shown in Figure II-I-14. Because the mutation occurred in only one parental gamete, the recurrence risk for other offspring of the parents remains very low. However, the recurrence risk for future offspring of the affected individual would be the same as that of any individual who has inherited the disease-causing mutation.

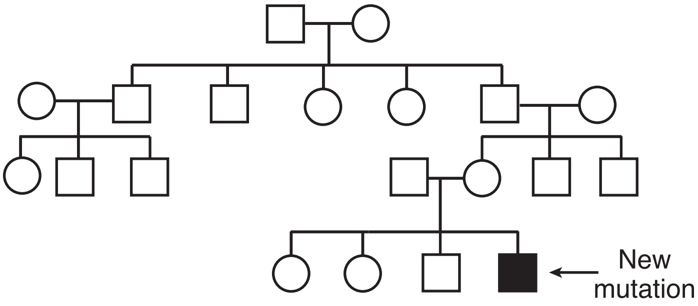


Figure II-1-14. Pedigree with a New Mutation

Delayed Age of Onset

Many individuals who carry a disease-causing mutation do not manifest the phenotype until later in life. This can complicate the interpretation of a pedigree because it may be difficult to distinguish genetically normal individuals from those who have inherited the mutation but have not yet displayed the phenotype.

Clinical Correlate

Diseases with Delayed Age of Onset

- Acute intermittent porphyria (peri- or postpubertal)
- Huntington disease
- Hemochromatosis
- Familial breast cancer

Delayed Age of Onset in Huntington Disease

Huntington disease, an autosomal dominant condition, affects approximately 1 in 20,000 individuals. Features of the disease include progressive dementia, loss of motor control, and affective disorder. This is a slowly progressing disease, with an average duration of approximately 15 years. Common causes of death include aspiration pneumonia, head trauma (resulting from loss of motor control), and suicide. Most patients first develop symptoms in their 30s or 40s, so this is a good example of a disease with delayed age of onset. The mutation produces a buildup of toxic protein aggregates in neurons, eventually resulting in neuronal death.

Anticipation

Anticipation refers to a pattern of inheritance in which individuals in the most recent generations of a pedigree develop a disease at an earlier age or with greater severity than do those in earlier generations. For a number of genetic diseases, this phenomenon can be attributed to the gradual expansion of trinucleotide repeat polymorphisms within or near a coding gene.

Huntington disease was cited above as an example of delayed age of onset; it is also a good example of anticipation.

The condition results from a gain-of-function mutation on chromosome 4 and is an example of a trinucleotide repeat expansion disorder. Normal *huntingin* genes have fewer than 27 CAG repeats in the 5′ coding region, and the number is stable from generation to generation. In families who eventually present with Huntington disease, premutations of 27–35 repeats are seen, although these individuals do not have Huntington disease. Some of these individuals (generally males) may then transmit an expanded number of repeats to their offspring. Individuals with more than 39 repeats are then seen, and these individuals develop symptoms. Within this group, age of onset is correlated with the number of repeats and ranges from a median age 66 (39 repeats) to age <20 (more than 70 repeats).

The figure below illustrates anticipation in a family with Huntington disease. The ages of onset for the affected individuals are shown along with the number of CAG repeats (in parentheses).

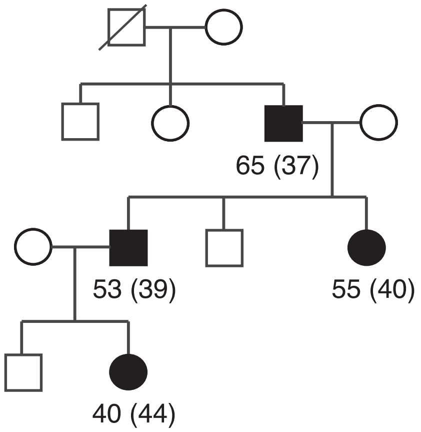


Figure II-1-15. Anticipation for Huntington Disease, an Autosomal Dominant Disorder

Table II-1-1. Diseases Showing Anticipation Associated with Triplet Repeat Expansions

Disease	Symptoms	Repeat
Huntington disease (autosomal dominant)	Movement abnormality Emotional disturbance Cognitive impairment Death 10–15 years after onset	CAG 5′ coding
Fragile X syndrome (X dominant)	Mental retardation Large ears and jaw Post-pubertal macro-orchidism (males) Attention deficit disorder (in females)	CGG 5′ UTR
Myotonic dystrophy (autosomal dominant)	Muscle loss Cardiac arrhythmia Testicular atrophy Frontal baldness Cataracts	CTG 3′ UTR
Friedreich ataxia (autosomal recessive)	Early onset progressive gait and limb ataxia Areflexia in all 4 limbs Hypertrophic cardiomyopathy Axonal sensory neuropathy Kyphoscoliosis	GAA Intron 1

Clinical Correlate

Friedreich Ataxia

A 16-year-old girl is seen by her neurologist for increasing weakness in her arms. She was apparently normal at birth, began walking at age 13 months, and had normal development until age 6. At that time her parents noted increasing clumsiness and stumbling. After undergoing neurologic testing, she was diagnosed with Friedreich ataxia. She began using a wheelchair at age 8 and currently cannot stand or walk unaided. She has now developed hypertrophic obstructive cardiomyopathy. She is breathless upon exertion but not at rest. She has kyphoscoliosis that has been progressive since age 12 but does not impair her breathing. Deep tendon reflexes are absent and there was an extensor plantar response bilaterally.

Friedreich ataxia is caused by expansion of a GAA repeat in the *frataxin* gene and is an autosomal recessive condition. Average life expectancy is approximately age 40, but can vary significantly.

Recall Question

Which of the following terms describes the occurrence of multiple organ systems being involved by a single disease-causing mutation?

A. Incomplete penetrance

B. Locus heterogeneity

C. Pleiotropy

D. Variable expression

Answer: C

Imprinting

High-Yield

Imprinting refers to the fact that a small number of genes are transcriptionally active only when transmitted by one of the two sexes. The homologous locus in the other parent is rendered transcriptionally inactive. Thus, for imprinted loci, it is normal to have only the maternal (for some loci) active, or only the paternal (for other loci) active.

Imprinting:

- Occurs during gametogenesis.
- Is maintained in all somatic cells of the offspring.
- During gametogenesis in the offspring, is erased and re-established according to the sex of the individual.
- Involves methylation and possibly other mechanisms to imprint or inactivate the appropriate loci.
- Occurs in specific loci on several chromosomes.

Prader-Willi and Angelman Syndromes. On rare occasion, the transcriptionally active gene may be deleted from the chromosome (perhaps by unequal crossover) during gametogenesis. This leaves the offspring with no active gene at that locus. The gene from one parent is inactivated due to normal imprinting, and the gene from the other parent deleted by a mutation. This situation may result in a genetic disease.

Prader-Will Syndrome

A 3-year-old boy is evaluated for obesity. At birth he fed poorly and was somewhat hypotonic and lethargic. At that time he was diagnosed with failure to thrive, cause unknown, and was given intragastric feedings until he regained his birth weight. He continued to gain weight slowly but remained in the lowest quartile for age-appropriate weight and height. Walking was delayed until he was age 26 months. Over the last year his appetite has increased dramatically. He has begun having temper tantrums of increasing frequency and violence, causing his withdrawal from preschool. His current evaluation reveals an obese boy with mental and developmental delay. The physician also notes underdeveloped genitalia, and she refers the boy to a genetics clinic for karyotype analysis. The result shows a deletion from one copy of chromosome 15q11-q13 consistent with Prader-Willi syndrome.

Prader-Willi syndrome is caused by loss from the paternal chromosome of an imprinted locus mapping to 15q11-13 that includes the gene *SNRPN*. This gene, normally active from the paternal copy of chromosome 15, encodes a component of mRNA splicing.

Interestingly, a different genetic disease—Angelman syndrome—is produced if there is a deletion of 15q11-13 from the maternal chromosome. In this case the locus imprinted in the maternal chromosome includes a gene involved in the ubiquitin pathway known as *UBE3A*, for which the maternal gene is normally expressed while the paternal gene is silenced. This has led to the conclusion that there are at least 2 imprinted genes within this region, one active on the paternal chromosome 15 and the other normally active on the maternal chromosome 15. Loss, usually by deletion of paternal 15q11-13, causes Prader-Willi, whereas loss of the maternal 15q11-13 causes Angelman syndrome (see margin notes on next page).

Uniparental Disomy

Uniparental disomy is a rare condition in which both copies of a particular chromosome are contributed by one parent. This may cause problems if the chromosome contains an imprinted region or a mutation. For example, 25–30% of Prader-Willi cases are caused by maternal uniparental disomy of chromosome 15. A smaller percentage of Angelman syndrome is caused by paternal uniparental disomy of chromosome 15.

Clinical Correlate

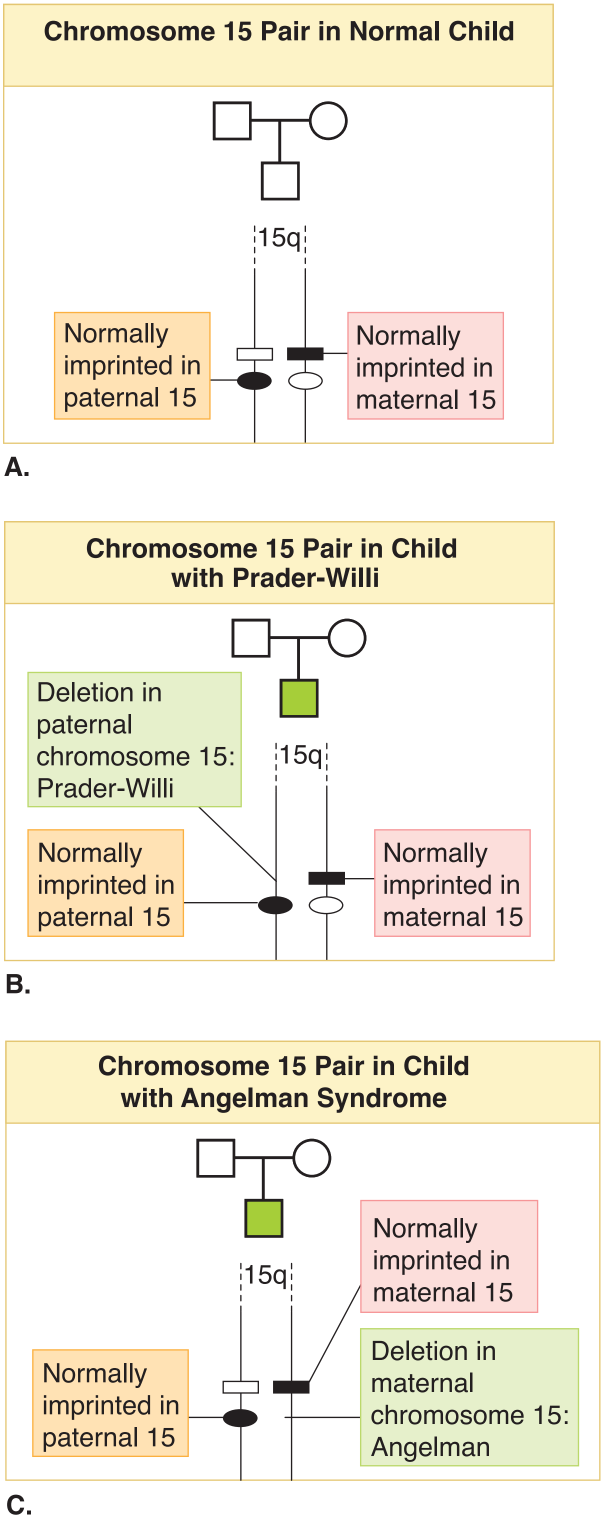
Prader-Willi Syndrome

- Affects males and females
- Neonatal hypotonia
- Poor feeding in neonatal period
- Behavior problems
- Moderate mental and developmental retardation
- Hypogonadism, underdeveloped genitalia
- Hyperphagia (overeating) and obesity by ages 2–4 years
- Small hands and feet
- Deletion from paternal 15q
- Very low recurrence risk

Clinical Correlate

Angelman Syndrome

- Affects males and females
- Severe mental retardation
- Seizures
- Ataxia
- Puppet-like posture of limbs
- Happy disposition
- Deletion from maternal 15q
- Very low recurrence risk



A. Loci normally imprinted on chromosome 15
B. Deletion causing Prader-Willi syndrome
C. Deletion causing Angelman syndrome

Figure II-1-16. Prader-Willi and Angelman Syndromes: Diseases Involving Imprinted Loci

Review Questions

1. A 25-year-old woman has mild expression of hemophilia A. A genetic diagnosis reveals that she is a heterozygous carrier of a mutation in the X-linked factor VIII gene. What is the most likely explanation for mild expression of the disease in this individual?

   A. A high proportion of the X chromosomes carrying the mutation are active in this woman
   B. Her father is affected, and her mother is a heterozygous carrier
   C. Nonsense mutation causing truncated protein
   D. One of her X chromosomes carries the SRY gene
   E. X inactivation does not affect the entire chromosome

2. A 20-year-old man has had no retinoblastomas but has produced two offspring with multiple retinoblastomas. In addition, his father had two retinoblastomas as a young child, and one of his siblings has had 3 retinoblastomas. What is the most likely explanation for the absence of retinoblastomas in this individual?

   A. A new mutation in the unaffected individual, which has corrected the disease-causing mutation
   B. Highly variable expression of the disorder
   C. Incomplete penetrance
   D. Multiple new mutations in other family members
   E. Pleiotropy

3. A 30-year-old man is phenotypically normal, but two of his siblings died from infantile Tay-Sachs disease, an autosomal recessive condition that is lethal by the age of 5. What is the risk that this man is a heterozygous carrier of the disease-causing mutation?

   A. 1/4
   B. 1/2
   C. 2/3
   D. 3/4
   E. Not elevated above that of the general population

4. A large, 3 generation family in whom multiple members are affected with a rare, undiagnosed disease is being studied. Affected males never produce affected children, but affected females do produce affected children of both sexes when they mate with unaffected males. What is the most likely mode of inheritance?

   A. Autosomal dominant, with expression limited to females
   B. Y-linked
   C. Mitochondrial
   D. X-linked dominant
   E. X-linked recessive

5. A man who is affected with hemophilia A (X-linked recessive) mates with a woman who is a heterozygous carrier of this disorder. What proportion of this couple's daughters will be affected, and what proportion of the daughters will be heterozygous carriers?

   A. 0%; 50%
   B. 100%; 0%
   C. 0%; 100%
   D. 50%; 50%
   E. 2/3; 1/3

6. The clinical progression of Becker muscular dystrophy is typically much slower than that of Duchenne muscular dystrophy. This is usually the result of

   A. gain-of-function mutations in the Duchenne form; loss-of-function mutations in the Becker form
   B. in-frame deletions or insertions in the Becker form; frameshift deletions or insertions in the Duchenne form
   C. mis-sense mutations in the Becker form; nonsense mutations in the Duchenne form
   D. mutations at two distinct loci for these two forms of muscular dystrophy
   E. nonsense mutations in the Becker form; missense mutations in the Duchenne form

7. A 10-year-old girl is diagnosed with Marfan syndrome, an autosomal dominant condition. An extensive review of her pedigree indicates no previous family history of this disorder. The most likely explanation for this pattern is

   A. highly variable expression of the disease phenotype
   B. incomplete penetrance
   C. mitochondrial compensation in the mother
   D. new mutation transmitted by one of the parents to the affected girl
   E. pleiotropy

8. In assessing a patient with osteogenesis imperfecta, a history of bone fractures, as well as blue sclerae, are noted. These findings are an example of

   A. allelic heterogeneity
   B. gain-of-function mutation
   C. locus heterogeneity
   D. multiple mutations
   E. pleiotropy

9. In studying a large number of families with a small deletion in a specific chromosome region, it is noted that the disease phenotype is distinctly different when the deletion is inherited from the mother as opposed to the father. What is the most likely explanation?

   A. Imprinting
   B. Mitochondrial inheritance
   C. Sex-dependent penetrance
   D. X-linked dominant inheritance
   E. X-linked recessive inheritance

10. A man and woman are both affected by an autosomal dominant disorder that has 80% penetrance. They are both heterozygotes for the disease-causing mutation. What is the probability that they will produce phenotypically normal offspring?

    A. 20%
    B. 25%
    C. 40%
    D. 60%
    E. 80%

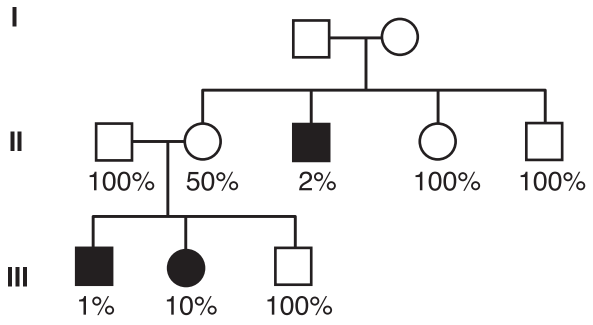
11. The severe form of alpha-1 antitrypsin deficiency is the result of a single nucleotide substitution that produces a single amino acid substitution. This is best described as a

    A. Frameshift mutation
    B. In-frame mutation
    C. Missense mutation
    D. Nonsense mutation
    E. Splice-site mutation

12. Waardenburg syndrome is an autosomal dominant disorder in which patients may exhibit a variety of clinical features, including patches of prematurely grey hair, white eyelashes, a broad nasal root, and moderate to severe hearing impairment. Occasionally, affected individuals display two eyes of different colors and a cleft lip and/or palate. Patients who possess a mutation in the *PAX3* gene on chromosome 2 can present with all of these disparate signs and symptoms. Which of the following characteristics of genetic traits is illustrated by this example?

    A. Anticipation
    B. Imprinting
    C. Incomplete penetrance
    D. Locus heterogeneity
    E. Pleiotropy

13. Hunter disease is an X-linked recessive condition in which a failure of mucopolysaccharide breakdown results in progressive mental retardation, deafness, skeletal abnormalities, and hepatosplenomegaly. In the family pedigree shown, all affected individuals were diagnosed biochemically by assaying activity of iduronate 2-sulfatase, the enzyme encoded by the gene involved in Hunter syndrome. Activity of the enzyme relative to the normal range is displayed below the symbol for selected individuals in the pedigree. What is the most likely explanation for the presence of the syndrome in individual III-2?



A. She is a manifesting heterozygote.

B. She is homozygous for the disease-producing allele.

C. She is not the daughter of II-1.

D. The trait has incomplete penetrance.

E. The trait has variable expression.

14. A 9-year-old boy is referred to a pediatric clinic by his school psychologist because of poor academic performance, cognitive delay, and hyperkinetic behavior. Family history is significant for early dementia and ataxia in the maternal father. Physical examination reveals that the boy has a long thin face with prominent ears, some facial asymmetry, and a prominent forehead and jaw. His vital signs are normal, his lungs are clear to auscultation, and heart sounds are normal. His abdomen is soft, nontender, and nondistended. Examination of the extremities showed hyperextensible finger joints. The examining physician suspects a possible genetic disorder. What would be the best test to diagnose this disease?

A. Brain MRI

B. Cytogenetic testing for fragile X

C. Developmental evaluation by a speech/language therapist

D. EEG

E. Measurement of testicular volume

F. Southern blot analysis

Answers

1. **Answer: A.** The most likely explanation for mild expression in a heterozygous carrier is that when X inactivation occurred in the affected individual, the random process happened to inactivate most of the X chromosomes that carried the normal version of the factor VIII gene. Thus, most of the active X chromosomes in this individual would carry the mutation and would not produce factor VIII, leading to a clinically expressed deficiency.

   If the woman's father is affected and her mother is a carrier (**choice B**), she has a 50% chance of being an affected homozygote, but her expression is more likely to be severe.

   A nonsense mutation (**choice C**) is likely to produce severe expression if it is inherited from both the mother and the father.

   The SRY gene (**choice D**) is involved in sex determination and would not affect factor VIII expression.

   Although it is true that X inactivation does not affect the entire X chromosome (**choice E**), it consistently affects the factor VIII gene and thus could not explain the status of this woman.

2. **Answer: C.** Because multiple family members are affected and because mutations at the retinoblastoma gene are known to be sometimes nonpenetrant, the man in question is most likely an obligate carrier of the mutation who did not experience a second mutation in this gene during his fetal development.

   A new mutation correcting the defect could occur (**choice A**), but then the man's two sons would both have experienced new mutations. The combination of 3 mutations affecting 3 different individuals in the family is highly unlikely.

   Variable expression (**choice B**) refers to differences in the severity of a disorder but does not refer to the complete absence of the disorder, which is incomplete penetrance.

   The number of affected individuals in this family (4) makes multiple new mutations in so many individuals extremely unlikely (**choice D**). Remember that inherited mutations are rare events.

   Pleiotropy is observed in retinoblastoma (**choice E**), in that mutation carriers can develop other cancers, such as osteosarcoma. This, however, does not explain the lack of a tumor in the 20-year-old man.

3. **Answer: C.** Because two of the man's siblings had Tay-Sachs disease, his parents must both be carriers. This clearly elevates his risk above the general population and excludes **choice E**. He is not affected, so this excludes **choice A**, which is the probability of inheriting two copies of the disease allele. His risk of inheriting one copy of the disease gene at conception is 1/2 (**choice B**). However, the fact that he is phenotypically normal at age 30 means that he cannot have inherited copies of the disease gene from both parents. Only 3 possibilities remain: Either he inherited no copies of the mutation, he inherited a copy from his father, or he inherited a copy from his mother. Each of these possibilities is equally likely, and two of them lead to heterozygosity. Thus, the risk that he is a carrier is 2/3.

4. **Answer: C.** This is a pattern expected of mitochondrial inheritance because only females transmit mitochondrial DNA to their offspring. Thus, an affected female can transmit the mutation to her offspring of both sexes, but an affected male cannot transmit it.

   Choice A is excluded because, although the disease is not transmitted by males, it is seen in them.

   Under Y-linked inheritance (**choice B**), affected males would transmit the mutation and would transmit it only to their sons.

   X-linked dominant inheritance (**choice D**) is excluded because affected males can transmit X-linked dominant mutations to their daughters.

   X-linked recessive inheritance (**choice E**) could explain this pattern because affected males typically produce only heterozygous carrier daughters and unaffected sons (unless they mate with a carrier female). However, affected homozygous females, who will produce affected sons, would produce an affected daughter only if they mated with an affected male.

5. **Answer: D.** Because the man transmits his X chromosome to all of his daughters, all of the daughters must carry at least one copy of the mutation. The mother will transmit a mutation-carrying X chromosome half the time and a normal X chromosome half the time. Thus, half of the daughters will be heterozygous carriers, and half will be affected homozygotes, having received a mutation from both parents.

6. **Answer: B.** In-frame deletions or insertions typically produce an altered protein product (dystrophin), but the alteration is mild enough so that Becker muscular dystrophy results. Frame-shifts usually produce a truncated protein because a stop codon is eventually encountered. The truncated protein is degraded, resulting in an absence of dystrophin and a more severe disease phenotype.

   Both types of muscular dystrophy are X-linked recessive mutations, making a gain-of-function highly unlikely for either type (**choice A**).

   Because approximately 2/3 of all mutations leading to these diseases are insertions or deletions, differences in single-base mutations (i.e., missense or nonsense mutations) would not be the most likely explanation, excluding **choice C** and **choice E**.

   These two forms of muscular dystrophy are known to be encoded by the same locus, so locus heterogeneity (**choice D**) is excluded.

7. **Answer: D.** For an autosomal dominant condition, the first occurrence in a family is usually the result of a new mutation that occurred in one of the gametes transmitted by a parent of the affected individual.

   Although variable expression (**choice A**) is a characteristic of this disease, other family members (including a parent) would be likely to manifest at least mild expression of the disorder.

   The penetrance of Marfan mutations is high, so it is highly unlikely that all other gene carriers in the family would be nonpenetrant carriers (**choice B**).

   Mitochondrial genes are not known to affect the expression of Marfan syndrome (**choice C**).

Marfan syndrome is an excellent example of pleiotropy (**choice E**), but this principle refers to the fact that a single mutation can affect multiple aspects of the phenotype, so it would not explain the pattern observed in this pedigree.

8. **Answer: E.** Pleiotropy refers to the multiple effects exerted by a single mutation and thus describes the two features observed in this patient.

   Allelic heterogeneity is observed in osteogenesis imperfecta (**choice A**), but allelic heterogeneity causes variable expression in patients and is not the principle described here.

   Osteogenesis imperfecta is a good example of a disease in which locus heterogeneity (**choice C**) is observed, but this principle refers to the fact that a mutation in either the type 1 procollagen gene on chromosome 7 or the type 1 procollagen gene on chromosome 17 can result in imperfect formation of the trimeric protein. This principle does not explain the co-occurrence of fractures and blue sclerae.

   A single mutation at either the chromosome 7 or chromosome 17 locus is sufficient to cause the disease, so multiple mutations (**choice D**) do not explain the pattern.

9. **Answer: A.** Imprinting refers to the differential transcriptional activity of genes inherited from the father versus the mother.

   Under mitochondrial inheritance (**choice B**), only an affected mother can transmit the disease phenotype; the offspring of affected males are always unaffected.

   The other modes of inheritance can influence the relative proportions of affected individuals who belong to one gender or the other (e.g., more affected males under X-linked recessive inheritance, more affected females under X-linked dominant inheritance), but they do not involve any differences in expression depending on the transmitting parent.

10. **Answer: C.** If both parents are heterozygotes, there is a 75% chance that their offspring will receive one or two copies of the disease-causing gene (i.e., a 50% chance that the offspring will receive one copy and a 25% chance that the offspring will receive two copies). With 80% penetrance, the probability that the offspring will be affected is 0.75×0.8, or 0.6 (60%). The probability that the offspring will be phenotypically normal is $1 - 0.60$, or 0.40 (40%).

11. **Answer: C.** A missense mutation results in the change of only a single amino acid.

    Frameshift mutations (**choice A**) are the result of the deletion or insertion of a series of nucleotides that are not a multiple of 3 (thus altering the reading frame). Although the insertion or deletion of a single nucleotide would produce a frameshift, it is highly unlikely that it would alter only a single amino acid. The shift in the reading frame typically alters a number of amino acids subsequent to the insertion or deletion site.

    An in-frame mutation (**choice B**) is the insertion or deletion of a multiple of 3 nucleotides, so this single-nucleotide substitution cannot be an in-frame mutation.

A nonsense mutation (**choice D**) is a single nucleotide substitution that produces a stop codon and thus truncation of the polypeptide. Therefore, it typically alters more than a single amino acid.

Splice-site mutations (**choice E**) occur at intron-exon boundaries and typically result in the loss of an exon or the inclusion of part of an intron in the coding sequence. Thus, more than a single amino acid would be altered in a typical splice-site mutation.

12. **Answer: E.** Pleiotropy refers to the appearance of apparently unrelated characteristics resulting from a single genetic defect. It is often the result of the presence of a single altered molecule in multiple locations in the body, so that the single mutation has effects in multiple organ systems. In Marfan syndrome, for example, a defect in the fibrillin gene causes manifestations of the disease in the eye, aorta, and joints.

    Anticipation (**choice A**) describes the finding that in some pedigrees, a disease trait occurs in earlier and earlier age groups as the generations progress. It is often a finding in pedigrees in which trinucleotide repeat expansions are linked to disease expression.

    Imprinting (**choice B**) refers to the selective inactivation of a gene in one of the parental sexes during gametogenesis. Males and females inactivate different regions on several autosomal chromosomes, so that the maternal or paternal source of such a chromosome may have different results in the progeny.

    Incomplete penetrance (**choice C**) indicates that a certain fraction of individuals with a disease-producing genotype develop no symptoms. An example is hemochromatosis in which 1/300 people in the United States have the disease-producing genotype, but only about 1/2,000 ever show symptoms of the disease.

    Locus heterogeneity (**choice D**) refers to the case in which a mutation in any one of several distinct genetic loci can result in a single disease phenotype. It is common in cases where a single molecule is composed of multiple subunits. The alteration of any one of the subunits results in the formation of a molecule with altered function; thus, several different mutations can yield the same phenotypic result.

13. **Answer: A.** X-linked recessive diseases should be expressed much more commonly in males than in females because males are hemizygous for the X chromosome (they have only one copy). In the pedigree shown, Individuals I-2 and II-2 are obligate carriers of the trait and have a 1 in 2 chance of transmitting the disease gene to their offspring. Male offspring who receive the X chromosome with the disease-causing allele will develop the disease (Individuals II-3 and III-1), and female offspring who receive the X chromosome with the disease-causing allele will be carriers of the trait. In most cases, the presence of a second, normal X chromosome in these female heterozygotes will prevent the expression of the disease. In some cases, however, inactivation of the normal X-chromosome may occur in an unusually high percentage of her cells. If this happens, most cells will have the X-chromosome with the mutation, and even though she is heterozygous she may manifest symptoms (manifesting heterozygote). That this is the case with III-2 is confirmed by finding

lower than expected activity of the enzyme. One would expect a heterozygote to have approximately 50% normal enzyme activity. This woman has only 10%.

She is not homozygous for the disease-producing allele (**choice B**). Her father is unaffected by this X-linked recessive trait and therefore necessarily has the normal allele. The woman has inherited the disease-causing allele from her mother but as a carrier should have 50% normal enzyme activity and should not show symptoms.

She is not the daughter of II-1 (**choice C**) is not the best answer because this X-linked recessive trait is clearly segregating normally in this family. In most genetic diseases, which are relatively rare, it would be uncommon for a person entering the family through marriage (a different, biologic father to replace Individual II-1) to have the same rare genetic trait that is being expressed in the family studied.

That the trait has incomplete penetrance (**choice D**) is not a valid answer because incomplete penetrance results in diminished numbers of affected individuals, not increased numbers as shown here. You are asked in this question to explain why the female in Generation III expresses the disease trait when she would not be expected to do so, not to explain why someone who does not have the disease trait lacks it.

Variable expression (**choice E**) refers to the situation in which individuals with the disease-producing genotype have varying degrees of phenotypic expression. Individual III-2 does not have the disease-producing genotype.

14. **Answer: F.** This patient has fragile X syndrome, which is the most common cause of inherited mental retardation and, after trisomy 21, is the second most common cause of genetically associated mental deficiencies. The genetic basis of this disease is a triplet (CGG) repeat expansion in the 5′ untranslated region of a gene (FMR-1) on the X chromosome. The standard diagnostic testing for fragile X syndrome uses molecular genetic techniques. The exact number of CGG triplet repeats can be determined by Southern blotting or by amplification of the repeat with a polymerase chain reaction and gel electrophoresis. Southern blot analysis provides a more accurate estimation of the number of CGG triplet repeats if a full mutation is present (with a large CGG expansion).

    Males with a permutation (moderate expansion but not sufficient to cause classic symptoms of fragile X) may have a fragile X tremor/ataxia syndrome (FXTAS) that presents later in life, usually after 50 years of age. Fragile X is also seen in females where learning disabilities and mild mental retardation characterize the syndrome.

    Brain MRI (**choice A**) and EEG (**choice D**) are not useful in diagnosis of fragile X syndrome, but may be indicated when patient presents with seizures.

    Fragile X chromosomes may show breakage when cultured in a medium containing folate; however, this cytogenetic testing for fragile X (**choice B**) is not as sensitive as molecular testing and cannot be considered as the best test with a false-negative result rate of approximately 20%.

Developmental evaluation by a speech/language therapist (**choice C**) will allow one to detect mental retardation; however, it does not help to establish the diagnosis of fragile X syndrome.

Measurement of testicular volume (**choice E**) may be helpful in postpubertal males when fragile X syndrome is suspected. In normal males, average testicular volume is 17 mL; in patients with fragile X syndrome, testicular volume is more than 25 mL and can be as high as 120 mL. However, measurement of testicular volume cannot be considered as a best diagnostic test, and this patient is only 9 years old.

Population Genetics 2

Learning Objectives

- ❏ Solve problems concerning genotype and allele frequencies
- ❏ Know how to apply the Hardy-Weinberg equilibrium
- ❏ Interpret scenarios about factors responsible for genetic variation in/among populations

DEFINITION

Population genetics is the study of genetic variation in populations. Basic concepts of population genetics allow us to understand how and why the prevalence of various genetic diseases differs among populations.

GENOTYPE AND ALLELE FREQUENCIES

An essential step in understanding genetic variation is to measure it in populations. This is done by estimating genotype and allele frequencies.

Genotype Frequencies

For a given locus, the genotype frequency measures the proportion of each genotype in a population. Suppose that a population of 100 individuals has been assayed for an autosomal restriction fragment length polymorphism. If the RFLP has 2 possible alleles, labeled 1 and 2, there are 3 possible genotypes: 1-1, 1-2, and 2-2.

Visualization of a Southern blot allows us to determine the genotype of each individual in our population, and we find that the genotypes are distributed as follows:

Table II-2-1. Genotype Frequency

Genotype	Count	Genotype Frequency
1-1	49	0.49
1-2	42	0.42
2-2	9	0.09
Total	100	1.00

The genotype frequency is then obtained by dividing the count for each genotype by the total number of individuals. Thus, the frequency of genotype 1-1 is 49/100 = 0.49, and the frequencies of genotypes 1-2 and 2-2 are 0.42 and 0.09, respectively.

Allele Frequencies

The allele frequency measures the proportion of chromosomes that contain a specific allele. To continue the earlier RFLP example, we wish to estimate the frequencies of alleles 1 and 2 in our population. Each individual with the 1-1 genotype has 2 copies of allele 1, and each heterozygote (1-2 genotype) has one copy of allele 1. Because each diploid somatic cell contains 2 copies of each autosome, our denominator is 200. Thus, the frequency of allele 1 in the population is:

$$\frac{(2 \times 49) + 42}{200} = 0.7$$

(The same approach can be used to estimate the frequency of allele 2, which is 0.3. A convenient shortcut is to remember that the allele frequencies for all of the alleles of a given locus must add up to 1. Therefore, we can obtain the frequency of allele 2 simply by subtracting the frequency of allele 1 (0.7) from 1.

Note

Genotype frequencies measure the proportion of each genotype in a population. Allele frequencies measure the proportion of chromosomes that contain a specific allele (of a gene).

HARDY-WEINBERG EQUILIBRIUM

If a population is large and if individuals mate at random with respect to their genotypes at a locus, the population should be in Hardy-Weinberg equilibrium. This means that there is a constant and predictable relationship between genotype frequencies and allele frequencies. This relationship, expressed in the Hardy-Weinberg equation, allows one to estimate genotype frequencies if one knows allele frequencies, and vice versa.

The Hardy-Weinberg Equation

$$p^2 + 2pq + q^2 = 1$$

In this equation:

- p = frequency of allele 1 (conventionally the most common, normal allele)
- q = frequency of allele 2 (conventionally a minor, disease-producing allele)
- p^2 = frequency of genotype 1-1 (conventionally homozygous normal)
- $2pq$ = frequency of genotype 1-2 (conventionally heterozygous)
- q^2 = frequency of genotype 2-2 (conventionally homozygous affected)

In most cases where this equation is used, a simplification is possible. Generally p, the normal allele frequency in the population, is very close to 1 (e.g., most of the alleles of this gene are normal). In this case, we may assume that $p \sim 1$, and the equation simplifies to:

$$1 + 2q + q^2 \sim 1$$

The frequency of the disease-producing allele in question, q, is a very small fraction. This simplification would not necessarily be used in actual medical genetics practice, but for answering test questions, it works quite well. However, if the disease prevalence >1/100, e.g., q >1/10, the complete Hardy-Weinberg equation should be used to obtain an accurate answer. In this case, $p = 1 - q$.

Although the Hardy-Weinberg equation applies equally well to autosomal dominant and recessive alleles, genotypes, and diseases, the equation is most frequently used with autosomal recessive conditions. In these instances, a large percentage of the disease-producing allele is "hidden" in heterozygous carriers who cannot be distinguished phenotypically (clinically) from homozygous normal individuals.

Practical Application of Hardy-Weinberg

High-Yield

A simple example is illustrated by the following case.

> A 20-year-old college student is taking a course in human genetics. She is aware that she has an autosomal recessive genetic disease that has required her lifelong adherence to a diet low in natural protein with supplements of tyrosine and restricted amounts of phenylalanine. She also must avoid foods artificially sweetened with aspartame (Nutrasweet™). She asks her genetics professor about the chances that she would marry a man with the disease-producing allele.

The geneticist tells her that the known prevalence of PKU in the population is 1/10,000 live births, but the frequency of carriers is much higher, approximately 1/50. Her greatest risk comes from marrying a carrier for two reasons. First, the frequency of carriers for this condition is much higher than the frequency of affected homozygotes, and second, an affected person would be identifiable clinically. The geneticist used the Hardy-Weinberg equation to estimate the carrier frequency from the known prevalence of the disease in the following way:

Disease prevalence = q^2 = 1/10,000 live births

Carrier frequency = 2q (to be calculated)

q = square root of 1/10,000, which is 1/100

2q = 2/100, or **1/50, the carrier frequency**

> The woman now asks a second question: "Knowing that I have a 1/50 chance of marrying a carrier of this allele, what is the probability that I will have a child with PKU?"

The geneticist answers, "The chance of you having a child with PKU is 1/100." This answer is based on the joint occurrence of two nonindependent events:

- The probability that she will marry a heterozygous carrier (1/50), and
- If he is a carrier, the probability that he will pass his PKU allele versus the normal allele to the child (1/2).

These probabilities would be multiplied to give:

- 1/50 × 1/2 = **1/100, the probability that she will have a child with PKU.**

Note

Hardy-Weinberg Equilibrium in Phenylketonuria (PKU)

- Prevalence of PKU is 1/10,000 live births
- Allele frequency = (1/10,000) = 1/100 = 0.01
- Carrier frequency = 2(1/100) = 1/50

Bridge to Statistics

If events are nonindependent, multiply the probability of one event by the probability of the second event, assuming that the first has occurred.

For example, what is the probability that the student's husband will pass the disease-producing allele to the child? It is the probability that he will be a carrier (1/50, event 1) multiplied by the probability that he will pass the disease-causing gene along (1/2, event 2), assuming he is a carrier.

Note

Assuming random mating, the Hardy-Weinberg principle specifies a predictable relationship between allele frequencies and genotype frequencies in populations. This principle can be applied to estimate the frequency of heterozygous carriers of an autosomal recessive mutation.

In summary, there are 3 major terms one usually works with in the Hardy-Weinberg equation applied to autosomal recessive conditions:

- q^2, the disease prevalence
- 2q, the carrier frequency
- q, the frequency of the disease-causing allele

When answering questions involving Hardy-Weinberg calculations, it is important to identify which of these terms has been given in the stem of the question and which term you are asked to calculate.

This exercise demonstrates two important points:

- The Hardy-Weinberg principle can be applied to estimate the prevalence of heterozygous carriers in populations when we know only the prevalence of the recessive disease.
- For autosomal recessive diseases, such as PKU, the prevalence of heterozygous carriers is much higher than the prevalence of affected homozygotes. In effect, the vast majority of recessive genes are hidden in the heterozygotes.

Hardy-Weinberg Equilibrium for Dominant Diseases

The calculations for dominant diseases must acknowledge that most of the affected individuals will be heterozygous. In this case, the prevalence is 2q. (One can again use the assumption that $p \sim 1$.) The term q^2 represents the prevalence of homozygous affected individuals who, although much less commonly seen, may have more severe symptoms. For example,

- 1/500 people in the United States have a form of LDL-receptor deficiency and are at increased risk for cardiovascular disease and myocardial infarction.
- Taking $2q = 1/500$, one can calculate that $q^2 = 1/10^6$, or one in a million live births are homozygous for the condition. These individuals have greatly elevated LDL-cholesterol levels, a much-higher risk for cardiovascular disease than heterozygotes, and are more likely to present with characteristic xanthomas, xanthelasmas, and corneal arcus.

In contrast, in Huntington disease (autosomal dominant), the number of triplet repeats correlates much more strongly with disease severity than does heterozygous or homozygous status.

Sex Chromosomes and Allele Frequencies

When considering X-linked recessive conditions, one must acknowledge that most cases occur in hemizygous males (xY). Therefore, q = disease-producing allele frequency but, paradoxically, it also equals the prevalence of affected males. Thus, the statement "1/10,000 males has hemophilia A" also gives the allele frequency for the disease-producing allele: 1/10,000.

- q^2 = prevalence of disease in females ($1/10^8$, or 1/100,000,000)
- 2q = prevalence of female carriers (1/5,000)

This exercise demonstrates that:

- As with autosomal recessive traits, the majority of X-linked recessive genes are hidden in female heterozygous carriers (although a considerable number of these genes are seen in affected males).
- X-linked recessive traits are seen much more commonly in males than in females.

FACTORS RESPONSIBLE FOR GENETIC VARIATION IN/AMONG POPULATIONS

Although human populations are typically in Hardy-Weinberg equilibrium for most loci, deviations from equilibrium can be produced by new mutations, the introduction of a new mutation into a population from outside (founder effect), nonrandom mating (for example, consanguinity), the action of natural selection, genetic drift, and gene flow. Although these factors are discussed independently, often more than one effect contributes to allele frequencies in a population.

Mutation

Mutation, discussed previously, is ultimately the source of all new genetic variation in populations. In general, mutation rates do not differ very much from population to population.

In some cases, a new mutation can be introduced into a population when someone carrying the mutation is one of the early founders of the community. This is referred to as a **founder effect**. As the community rapidly expands through generations, the frequency of the mutation can be affected by natural selection, by genetic drift (*see* below), and by consanguinity.

Branched Chain Ketoacid Dehydrogenase Deficiency

Branched chain ketoacid dehydrogenase deficiency (maple syrup urine disease) occurs in 1/176 live births in the Mennonite community of Lancastershire, Pennsylvania. In the U.S. population at large, the disease occurs in only 1/180,000 live births. The predominance of a single mutation (allele) in the branched chain dehydrogenase gene in this group suggests a common origin of the mutation. This may be due to a founder effect.

Natural Selection

Natural selection acts upon genetic variation, increasing the frequencies of alleles that promote survival or fertility (referred to as fitness) and decreasing the frequencies of alleles that reduce fitness. The reduced fitness of most disease-producing alleles helps explain why most genetic diseases are relatively rare. Dominant diseases, in which the disease-causing allele is more readily exposed to the effects of natural selection, tend to have lower allele frequencies than do recessive diseases, where the allele is typically hidden in heterozygotes.

Note

The 4 evolutionary factors responsible for genetic variation in populations are:

- Mutation
- Natural selection
- Genetic drift
- Gene flow

Sickle Cell Disease and Malaria

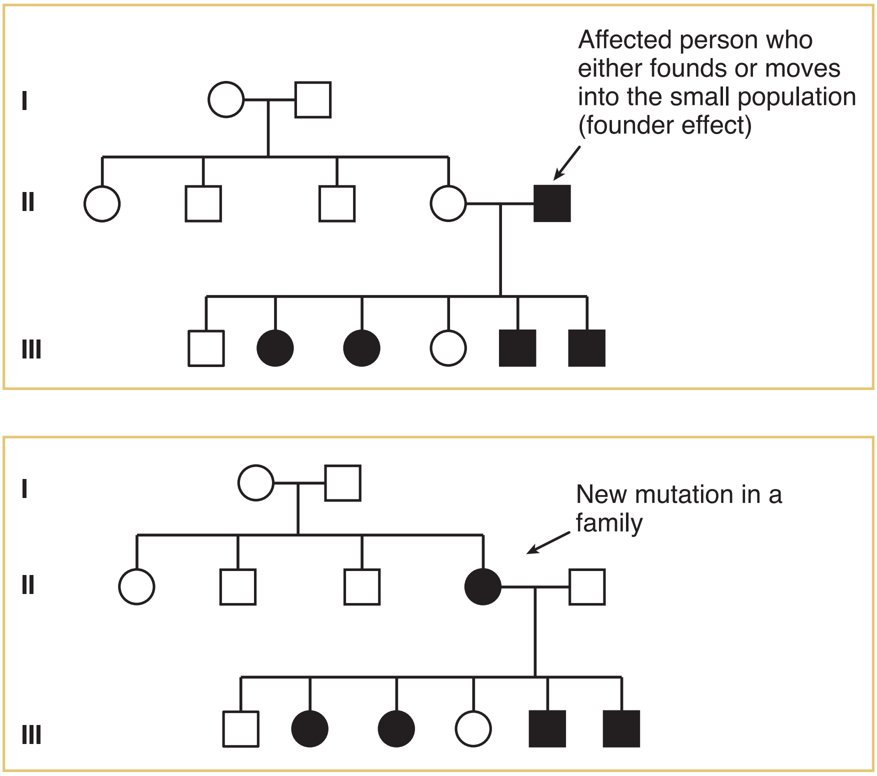
Sickle cell disease affects 1/600 African Americans and up to 1/50 individuals in some parts of Africa. How could this highly deleterious disease-causing mutation become so frequent, especially in Africa? The answer lies in the fact that the falciparum malaria parasite, which has been common in much of Africa, does not survive well in the erythrocytes of sickle cell heterozygotes. These individuals, who have no clinical signs of sickle cell disease, are thus protected against the lethal effects of malaria. Consequently, there is a heterozygote advantage for the sickle cell mutation, and it maintains a relatively high frequency in some African populations.

There is now evidence for heterozygote advantages for several other recessive diseases that are relatively common in some populations. Examples include:

- Cystic fibrosis (heterozygote resistance to typhoid fever)
- Hemochromatosis (heterozygote advantage in iron-poor environments)
- Glucose-6-phosphate dehydrogenase deficiency, hemolytic anemia (heterozygote resistance to malaria)

Genetic Drift

Mutation rates do not vary significantly from population to population, although they can result in significant differences in allele frequencies when they occur in small populations or are introduced by a founder effect. Mutation rates and founder effects act along with genetic drift to make certain genetic diseases more common (or rarer) in small, isolated populations than in the world at large. Consider the pedigrees (very small populations) shown in Figure II-2-1.



Genetic drift begins. In both examples the frequency of affected persons in generation III is 2/3, higher than the 1/2 predicted by statistics.

Figure II-2-1. Genetic Drift in Two Small Populations (Illustrated with a Dominant Disease)

If the woman and the affected man (II-5) in the top panel had 1,000 children rather than 6, the prevalence of the disease in their offspring (Generation III) would be closer to 1/2, the statistical mean. Although genetic drift affects populations larger than a single family, this example illustrates two points:

- When a new mutation or a founder effect occurs in a small population, genetic drift can make the allele more or less prevalent than statistics alone would predict.
- A relatively large population in Hardy-Weinberg equilibrium for an allele or many alleles can be affected by population "bottlenecks" in which natural disaster or large-scale genocide dramatically reduces the size of the population. Genetic drift may then change allele frequencies and a new Hardy-Weinberg equilibrium is reached.

Gene Flow

Gene flow refers to the exchange of genes among populations. Because of gene flow, populations located close to one another often tend to have similar gene frequencies. Gene flow can also cause gene frequencies to change through time: The frequency of sickle cell disease is lower in African Americans in part because of gene flow from other sectors of the U.S. population that do not carry the disease-causing mutation; in addition, the heterozygote advantage for the sickle cell mutation (*see* text box) has disappeared because malaria has become rare in North America.

Note

Consanguineous matings are more likely to produce offspring affected with recessive diseases because individuals who share common ancestors are more liable to share disease-causing mutations.

Consanguinity and Its Health Consequences

Consanguinity refers to the mating of individuals who are related to one another (typically, a union is considered to be consanguineous if it occurs between individuals related at the second-cousin level or closer). Figure II-2-2 illustrates a pedigree for a consanguineous union. Because of their mutual descent from common ancestors, relatives are more likely to share the same disease-causing genes. Statistically,

- Siblings (II-2 and II-3 or II-4) share 1/2 of their genes.
- First cousins (III-3 and III-4) share 1/8 of their genes (1/2 × 1/2 × 1/2).
- Second cousins (IV-1 and IV-2) share 1/32 of their genes (1/8 × 1/2 × 1/2).

These numbers are referred to as the coefficients of relationship. Thus, if individual III-1 carries a disease-causing allele, there is a 1/2 chance that individual III-3 (his brother) has it and a 1/8 chance that individual III-4 (his first cousin) has it.

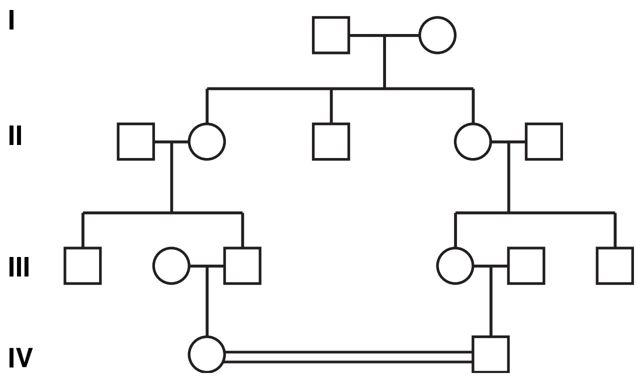


Figure II-2-2. A Pedigree Illustrating Consanguinity

Consequently, there is an increased risk of genetic disease in the offspring of consanguineous matings. Dozens of empirical studies have examined the health consequences of consanguinity, particularly first-cousin matings. These studies show that the offspring of first-cousin matings are approximately twice as likely to present with a genetic disease as are the offspring of unrelated matings. The frequency of genetic disease increases further in the offspring of closer unions (e.g., uncle/niece or brother/sister matings).

Review Questions

1. A population has been assayed for a 4-allele polymorphism, and the following genotype counts have been obtained:

Genotype	Count
1,1	4
1,3	8
1,4	3
2,3	5
2,4	9
3,3	4
3,4	6
4,4	11

On the basis of these genotype counts, what are the gene frequencies of alleles 1 and 2?

A. 0.38, 0.28
B. 0.19, 0.14
C. 0.095, 0.07
D. 0.25, 0.25
E. 0.38, 0.20

2. Which of the following best characterizes Hardy-Weinberg equilibrium?

A. Consanguinity has no effect on Hardy-Weinberg equilibrium.
B. Genotype frequencies can be estimated from allele frequencies, but the reverse is not true.
C. Natural selection has no effect on Hardy-Weinberg equilibrium.
D. Once a population deviates from Hardy-Weinberg equilibrium, it takes many generations to return to equilibrium.
E. The frequency of heterozygous carriers of an autosomal recessive mutation can be estimated if one knows the incidence of affected homozygotes in the population.

3. In a genetic counseling session, a healthy couple has revealed that they are first cousins and that they are concerned about health risks for their offspring. Which of the following best characterizes these risks?

   A. Because the couple shares approximately half of their genes, most of the offspring are likely to be affected with some type of genetic disorder.
   B. The couple has an increased risk of producing a child with an autosomal dominant disease.
   C. The couple has an increased risk of producing a child with an autosomal recessive disease.
   D. The couple has an increased risk of producing a child with Down syndrome.
   E. There is no known increase in risk for the offspring.

4. An African American couple has produced two children with sickle cell disease. They have asked why this disease seems to be more common in the African American population than in other U.S. populations. Which of the following factors provides the best explanation?

   A. Consanguinity
   B. Genetic drift
   C. Increased gene flow in this population
   D. Increased mutation rate in this population
   E. Natural selection

5. If the incidence of cystic fibrosis is 1/2,500 among a population of Europeans, what is the predicted incidence of heterozygous carriers of a cystic fibrosis mutation in this population?

   A. 1/25
   B. 1/50
   C. 2/2,500
   D. 1/2,500
   E. $(1/2{,}500)^2$

6. A man is a known heterozygous carrier of a mutation causing hyperprolinemia, an autosomal recessive condition. Phenotypic expression is variable and ranges from high urinary excretion of proline to neurologic manifestations including seizures. Suppose that 0.0025% (1/40,000) of the population is homozygous for the mutation causing this condition. If the man mates with somebody from the general population, what is the probability that he and his mate will produce a child who is homozygous for the mutation involved?

   A. 1% (1/100)
   B. 0.5% (1/200)
   C. 0.25% (1/400)
   D. 0.1% (1/1,000)
   E. 0.05% (1/2,000)

7. The incidence of Duchenne muscular dystrophy in North America is about 1/3,000 males. On the basis for this figure, what is the gene frequency of this X-linked recessive mutation?

   A. 1/3,000

   B. 2/3,000

   C. $(1/3{,}000)^2$

   D. 1/6,000

   E. 1/9,000

Answers

1. **Answer: B.** The denominator of the gene frequency is 100, which is obtained by adding the number of genotyped individuals (50) and multiplying by 2 (because each individual has two alleles at the locus). The numerator is obtained by counting the number of alleles of each type: the 4 homozygotes with the 1,1 genotype contribute 8 copies of allele 1; the 1,3 heterozygotes contribute another 8 alleles; and the 1,4 heterozygotes contribute 3 alleles. Adding these together, we obtain 19 copies of allele 1. Dividing by 100, this yields a gene frequency of 0.19 for allele 1. For allele 2, there are two classes of heterozygotes that have a copy of the allele: those with the 2,3 and 2,4 genotypes. These 2 genotypes yield 5 and 9 copies of allele 2, respectively, for a frequency of 14/100 = 0.14.

2. **Answer: E.** The incidence of affected homozygotes permits the estimation of the frequency of the recessive mutation in the population. Using the Hardy-Weinberg equilibrium relationship between gene frequency and genotype frequency, the gene frequency can then be used to estimate the frequency of the heterozygous genotype in the population.

   Consanguinity (**choice A**) affects Hardy-Weinberg equilibrium by increasing the number of homozygotes in the population above the equilibrium expectation (i.e., consanguinity results in a violation of the assumption of random mating).

   Genotype frequencies can be estimated from gene frequencies (**choice B**), but gene frequencies can also be estimated from genotype frequencies (as in **choice A**).

   By eliminating a specific genotype from the population (e.g., affected homozygotes), natural selection can cause deviations from equilibrium (**choice C**).

   Only one generation of random mating is required to return a population to equilibrium (**choice D**).

3. **Answer: C.** Because the couple shares common ancestors (i.e., one set of grandparents), they are more likely to be heterozygous carriers of the same autosomal recessive disease-causing mutations. Thus, their risk of producing a child with an autosomal recessive disease is elevated above that of the general population.

   First cousins share approximately 1/8 of their genes, not 1/2 (**choice A**).

   Because both members of the couple are healthy, neither one is likely to harbor a dominant disease-causing mutation (**choice B**). In addition, consanguinity itself does not elevate the probability of producing a child with a dominant disease because only one copy of the disease-causing allele is needed to cause the disease.

   Down syndrome (**choice D**) typically is the result of a new mutation. When it is transmitted by an affected female, it acts like a dominant mutation and thus would not be affected by consanguinity.

   Empirical studies indicate that the risk of genetic disease in the offspring of first cousin couples is approximately double that of the general population (**choice E**).

4. **Answer: E.** The frequency of sickle cell disease is elevated in many African populations because heterozygous carriers of the sickle cell mutation are resistant to malarial infection but do not develop sickle cell disease, which is autosomal recessive. Thus, there is a selective advantage for the mutation in heterozygous carriers, elevating its frequency in the population.

   Consanguinity (**choice A**) could elevate the incidence of this autosomal recessive disease in a specific family, but it does not account for the elevated incidence of this specific disease in the African American population in general.

   The African American population is large and consequently would not be expected to have experienced elevated levels of genetic drift (**choice B**).

   Although there has been gene flow (**choice C**) from other populations into the African American population, this would be expected to decrease, rather than increase, the frequency of sickle cell disease because the frequency of this disease is highest in some African populations.

   There is no evidence that the mutation rate (**choice D**) is elevated in this population. In contrast, the evidence for natural selection is very strong.

5. **Answer: A.** This answer is obtained by taking the square root of the incidence (i.e., the frequency of affected homozygotes) to get a gene frequency for the disease-causing mutation (q) of 1/50 (0.02). The carrier frequency is given by 2pq, or approximately 2q, or 1/25.

6. **Answer: C.** One must first determine the probability that the man's mate will also be a heterozygous carrier. If the frequency of affected homozygotes (q^2) is 1/40,000, then the allele frequency, q, is 1/200. The carrier frequency in the population (approximately 2q) is 1/100. Three independent events must happen for their child to be homozygous for the mutation. The mate must be a carrier (probability 1/100), the mate must pass along the mutant allele (probability 1/2), and the man must also pass along the mutant allele (probability 1/2). Multiplying the 3 probabilities to determine the probability of their joint occurrence gives $1/100 \times 1/2 \times 1/2 = 1/400$.

7. **Answer: A.** Because males have only a single X chromosome, each affected male has one copy of the disease-causing recessive mutation. Thus, the incidence of an X-linked recessive disease in the male portion of a population is a direct estimate of the gene frequency in the population.

Cytogenetics 3

Learning Objectives

- ❑ Interpret scenarios about basic definitions and terminology
- ❑ Solve problems concerning numerical chromosome abnormalities
- ❑ Demonstrate understanding of structural chromosome abnormalities
- ❑ Solve problems concerning advances in molecular cytogenetics

OVERVIEW

This chapter reviews diseases that are caused by microscopically observable alterations in chromosomes. These alterations may involve the presence of extra chromosomes or the loss of chromosomes. They may also consist of structural alterations of chromosomes. Chromosome abnormalities are seen in approximately 1 in 150 live births and are the leading known cause of mental retardation. The vast majority of fetuses with chromosome abnormalities are lost prenatally: Chromosome abnormalities are seen in 50% of spontaneous fetal losses during the first trimester of pregnancy, and they are seen in 20% of fetuses lost during the second trimester. Thus, chromosome abnormalities are the leading known cause of pregnancy loss.

Note

X chromosome contains ~1,200 genes

Y chromosome contains ~50 genes

BASIC DEFINITIONS AND TERMINOLOGY

Karyotype

Chromosomes are most easily visualized during the metaphase stage of mitosis, when they are maximally condensed. They are photographed under the microscope to create a karyotype, an ordered display of the 23 pairs of human chromosomes in a typical somatic cell (Figure II-3-1). In Figure II-3-1A, a karyogram represents a drawing of each type of chromosome; the presentation is haploid (only one copy of each chromosome is shown). Figure II-3-1B is a karyotype of an individual male. It is diploid, showing both copies of each autosome, the X and the Y chromosome. Chromosomes are ordered according to size, with the sex chromosomes (X and Y) placed in the lower right portion of the karyotype.

Metaphase chromosomes can be grouped according to size and to the position of the centromere, but accurate identification requires staining with one of a variety of dyes to reveal characteristic banding patterns.

Chromosome banding

To visualize chromosomes in a karyotype unambiguously, various stains are applied so that banding is evident.

- **G-banding.** Mitotic chromosomes are partially digested with trypsin (to digest some associated protein) and then stained with Giemsa, a dye that binds DNA.

G-banding reveals a pattern of light and dark (G-bands) regions that allow chromosomes to be accurately identified in a karyotype. There are several other stains that can be used in a similar manner. The chromosomes depicted in Figure II-3-1 have been stained with Giemsa.

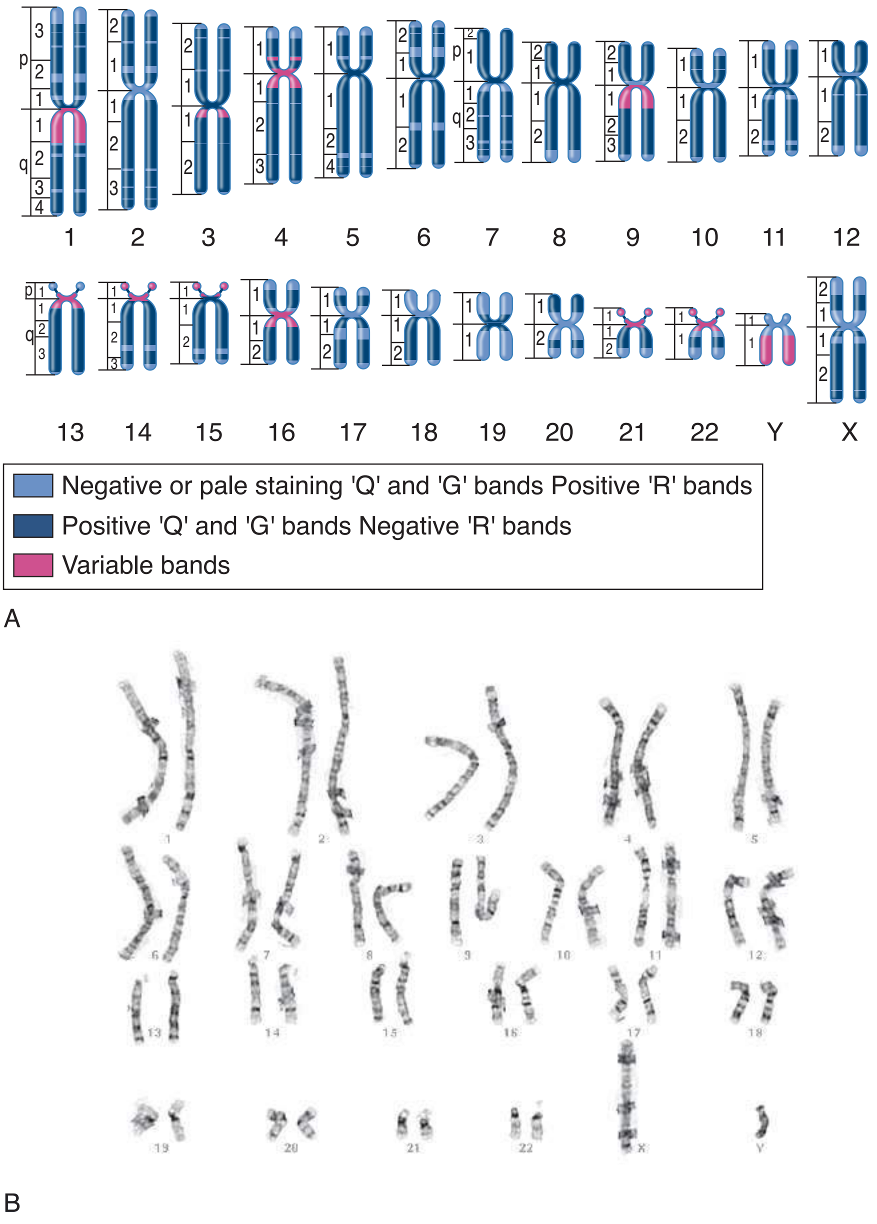


Figure II-3-1. Human Metaphase Chromosomes. (*A*) Idealized Drawing (Karyogram) and (*B*) Photograph of Metaphase Chromosomes (Karyotype)

Chromosome abnormalities in some cases can be identified visually by looking at the banding pattern, but this technique reveals differences (for instance, larger deletions) only to a resolution of about 4 Mb. Smaller abnormalities (microdeletions) must be identified in other ways (FISH), discussed at the end of the chapter.

Chromosome nomenclature

Each mitotic chromosome contains a centromere and two sister chromatids because the cell has gone through interphase and has entered mitosis when the karyotype analysis is performed (metaphase). The long arm of the chromosome is labeled q, and the short arm is labeled p. One of the characteristics described is the relative position of the centromere.

- **Metacentric** chromosomes (for instance, chromosome 1) have the centromere near the middle. The p and q arms are of roughly equal length.
- **Submetacentric** chromosomes have the centromere displaced toward one end (for example, chromosome 4). The p and q arms are evident.
- **Acrocentric** chromosomes have the centromere far toward one end. In these chromosomes, the p arm contains little genetic information, most of it residing on the q arm. Chromosomes 13, 14, 15, 21, and 22 are the acrocentric chromosomes. Only the acrocentric chromosomes are involved in
- **Robertsonian translocations**, which will be discussed in this chapter.

The tips of the chromosomes are called telomeres.

Table II-3-1 contains some standard nomenclature applied to chromosomes.

Table II-3-1. Common Symbols Used in Karyotype Nomenclature

1-22	Autosome number
X, Y	Sex chromosomes
(+) or (–)	When placed before an autosomal number, indicates that chromosome is extra or missing
p	Short arm of the chromosome
q	Long arm of the chromosome
t	Translocation
del	Deletion

NUMERICAL CHROMOSOME ABNORMALITIES

Euploidy

High-Yield

When a cell has a multiple of 23 chromosomes, it is said to be **euploid**. Gametes (sperm and egg cells) are euploid cells that have 23 chromosomes (one member of each pair); they are said to be **haploid**. Most somatic cells are **diploid**, containing both members of each pair, or 46 chromosomes.

Note

Euploid Cells (multiple of 23 chromosomes)

- Haploid (23 chromosomes): gametes
- Diploid (46 chromosomes): most somatic cells
- Triploid (69 chromosomes): rare lethal condition
- Tetraploid (92 chromosomes): very rare lethal condition

Two types of euploid cells with abnormal numbers of chromosomes are seen in humans: triploidy and tetraploidy.

Triploidy refers to cells that contain 3 copies of each chromosome (69 total). Triploidy, which usually occurs as a result of the fertilization of an ovum by 2 sperm cells, is common at conception, but the vast majority of these conceptions are lost prenatally. However, about 1 in 10,000 live births is a triploid. These babies have multiple defects of the heart and central nervous system, and they do not survive.

Tetraploidy refers to cells that contain 4 copies of each chromosome (92 total). This lethal condition is much rarer than triploidy among live births: Only a few cases have been described.

Aneuploidy

High-Yield

Aneuploidy, a deviation from the euploid number, represents the gain (+) or loss (–) of a specific chromosome. Two major forms of aneuploidy are observed:

- Monosomy (loss of a chromosome)
- Trisomy (gain of a chromosome)

Autosomal aneuploidy

Two generalizations are helpful:

- All autosomal monosomies are inconsistent with a live birth.
- Only 3 autosomal trisomies (trisomy 13, 18, and 21) are consistent with a live birth.

Trisomy 21 (47,XY,+21 or 47,XX,+21); Down Syndrome

- Most common autosomal trisomy
- Mental retardation
- Short stature
- Hypotonia
- Depressed nasal bridge, upslanting palpebral fissures, epicanthal fold
- Congenital heart defects in approximately 40% of cases
- Increased risk of acute lymphoblastic leukemia
- Alzheimer disease by fifth or sixth decade (*amyloid precursor protein, APP* gene on chromosome 21)
- Reduced fertility
- Risk increases with increased maternal age

Trisomy 18 (47,XY,+18 or 47,XX,+18); Edward Syndrome

- Clenched fist with overlapping fingers
- Inward turning, "rocker-bottom" feet
- Congenital heart defects
- Low-set ears, micrognathia (small lower jaw)
- Mental retardation
- Very poor prognosis

Trisomy 13 (47,XY,+13 or 47,XX,+13); Patau Syndrome

- Polydactyly (extra fingers and toes)
- Cleft lip, palate
- Microphthalmia (small eyes)
- Microcephaly, mental retardation
- Cardiac and renal defects
- Very poor prognosis

Sex chromosome aneuploidy

Aneuploidy involving the sex chromosomes is relatively common and tends to have less severe consequences than does autosomal aneuploidy. Some generalizations are helpful:

- At least one X chromosome is required for survival.
- If a Y chromosome is present, the phenotype is male (with minor exceptions).
- If more than one X chromosome is present, all but one will become a Barr body in each cell.

The two important sex chromosome aneuploidies are Turner syndrome and Klinefelter syndrome.

Klinefelter Syndrome (47,XXY)

- Testicular atrophy
- Infertility
- Gynecomastia
- Female distribution of hair
- Low testosterone
- Elevated FSH and LH
- High-pitched voice

Turner Syndrome (45,X or 45,XO)

- Only monosomy consistent with life
- 50% are 45,X
- Majority of others are mosaics for 45,X and one other cell lineage (46,XX, 47,XXX, 46,XY)
- Females with 45,X;46,XY are at increased risk for gonadal blastoma.
- Short stature
- Edema of wrists and ankles in newborn
- Cystic hygroma *in utero* resulting in excess nuchal skin and "webbed" neck
- Primary amenorrhea
- Coarctation of the aorta or other congenital heart defect in some cases
- Infertility
- Gonadal dysgenesis

Note

Trisomy is the most common genetic cause of spontaneous loss of pregnancy.

Note

Genetic Mosaicism in Turner Syndrome

Genetic mosaicism is defined as a condition in which there are cells of different genotypes or chromosome constitutions within a single individual. Some women with Turner syndrome have somatic cells that are 45,X and others that are 46,XX or 47,XXX. Mosaicism in Turner syndrome is thought to arise in early embryogenesis by mechanisms that are not completely understood.

Nondisjunction is the usual cause of aneuploidies

Germ cells undergo meiosis to produce the haploid egg or sperm. Normal meiosis is illustrated in Figure II-3-2A. The original cell is diploid for all chromosomes, although only one homologous pair is shown in the figure for simplicity. The same events would occur for each pair of homologs within the cell.

Figure II-3-2B shows the result of nondisjunction of one homologous pair (for example, chromosome 21) during meiosis 1. All other homologs segregate (disjoin) normally in the cell. Two of the gametes are diploid for chromosome 21. When fertilization occurs, the conception will be a trisomy 21 with Down syndrome. The other gametes with no copy of chromosome 21 will result in conceptions that are monosomy 21, a condition incompatible with a live birth.

Figure II-3-2C shows the result of nondisjunction during meiosis 2. In this case, the sister chromatids of a chromosome (for example, chromosome 21) fail to segregate (disjoin). The sister chromatids of all other chromosomes segregate normally. One of the gametes is diploid for chromosome 21. When fertilization occurs, the conception will be a trisomy 21 with Down syndrome. One gamete has no copy of chromosome 21 and will result in a conception that is a monosomy 21. The remaining two gametes are normal haploid ones.

Some important points to remember:

- Nondisjunction is the usual cause of aneuploidies including Down, Edward, Patau, Turner, and Klinefelter syndromes.
- Nondisjunction is more likely to occur during oogenesis than during spermatogenesis.
- Nondisjunction is more likely with increasing maternal age. Environmental agents (e.g., radiation, alcohol) appear to have no measurable influence.
- Nondisjunction is more likely in meiosis I than meiosis II.

Clinical Correlate: Maternal Age and Risk of Down Syndrome

Surveys of babies with trisomy 21 show that 90–95% of the time, the extra copy of the chromosome is contributed by the mother (similar figures are obtained for trisomies of the 18th and 13th chromosomes). The increased risk of Down syndrome with maternal age is well documented.

- For women age <30 the risk of bearing a child with Down is $<1/1{,}000$.
- At age 35 the risk increases to about 1/400.
- At age 40 the risk increases to 1/100.
- At age ≥ 45 the risk increases to about 1/25.

This increase reflects an elevated rate of nondisjunction in older ova (recall that all of a woman’s egg cells are formed during her fetal development and they remain suspended in prophase I until ovulation).

There is no corresponding increase in risk with advanced paternal age; sperm cells are generated continuously throughout the life of the male.

The increased risk of trisomy with advanced maternal age motivates >50% of pregnant women in North America to undergo prenatal diagnosis (typically amniocentesis or chorionic villus sampling. Down syndrome can also be screened by assaying maternal serum levels of α-fetoprotein, chorionic gonadotropin, and unconjugated estriol. This so-called *triple screen* can detect approximately 70% of fetuses with Down.

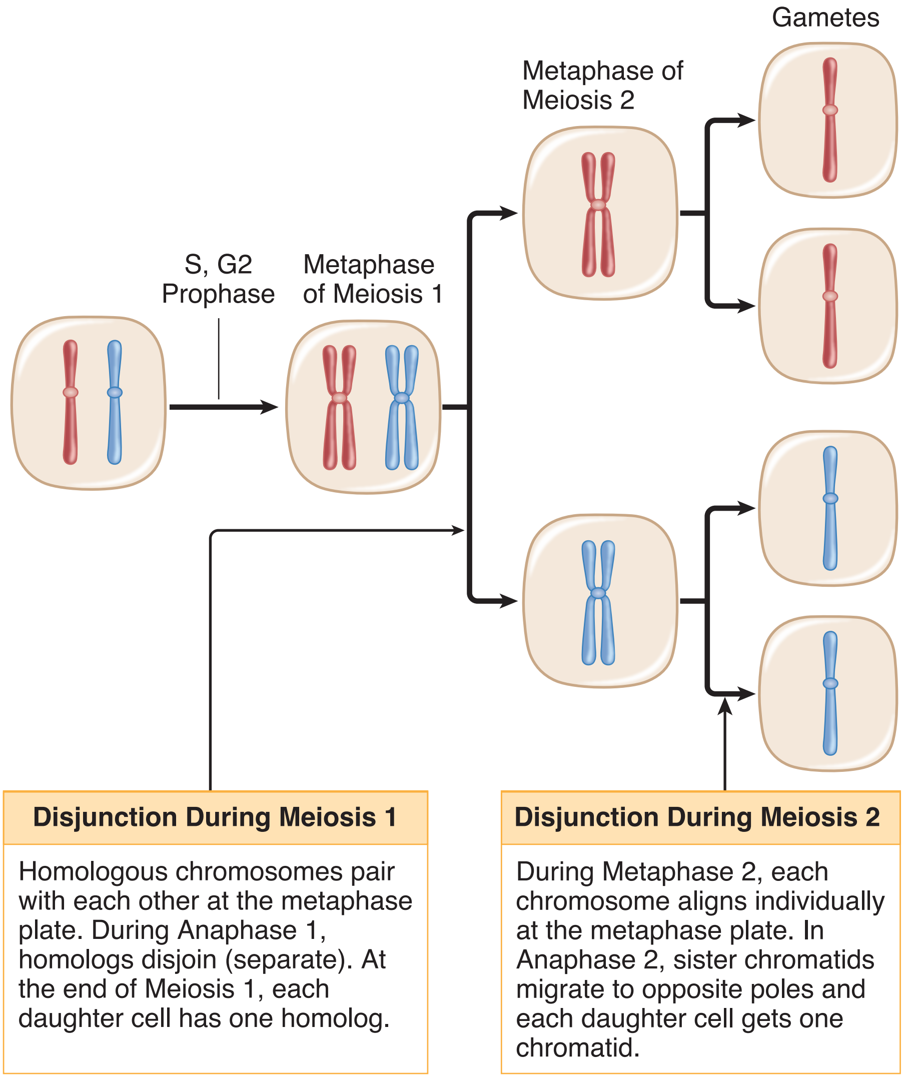


Figure II-3-2A. Disjunction During Normal Meiosis

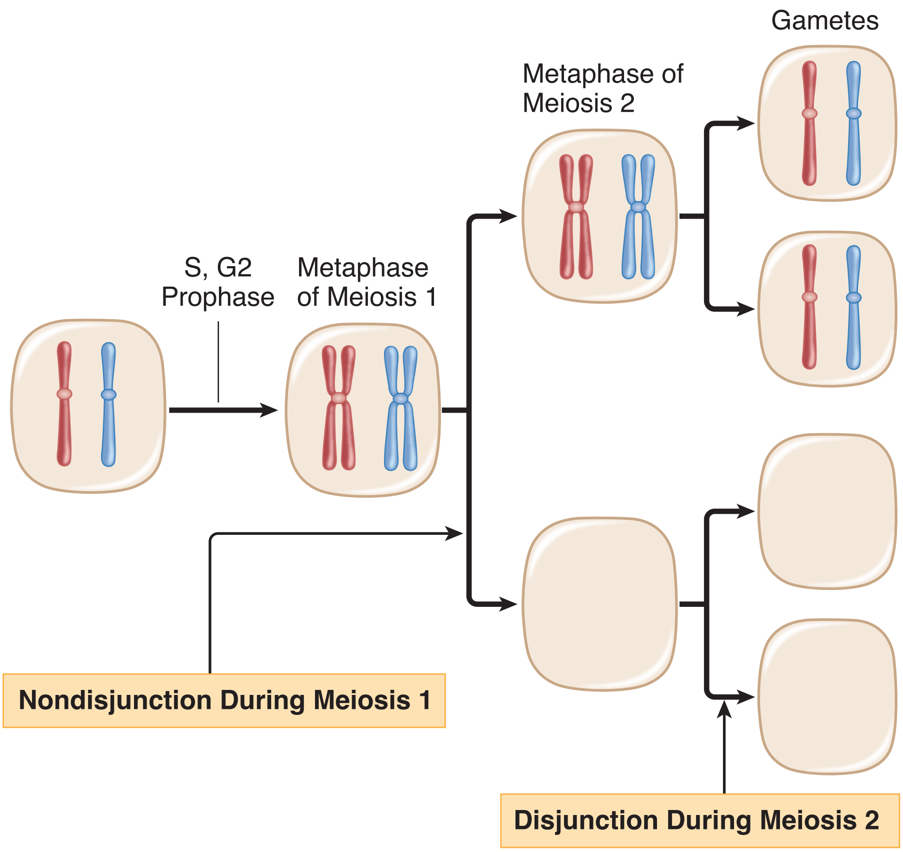


Figure II-3-2B. Nondisjunction During Meiosis 1

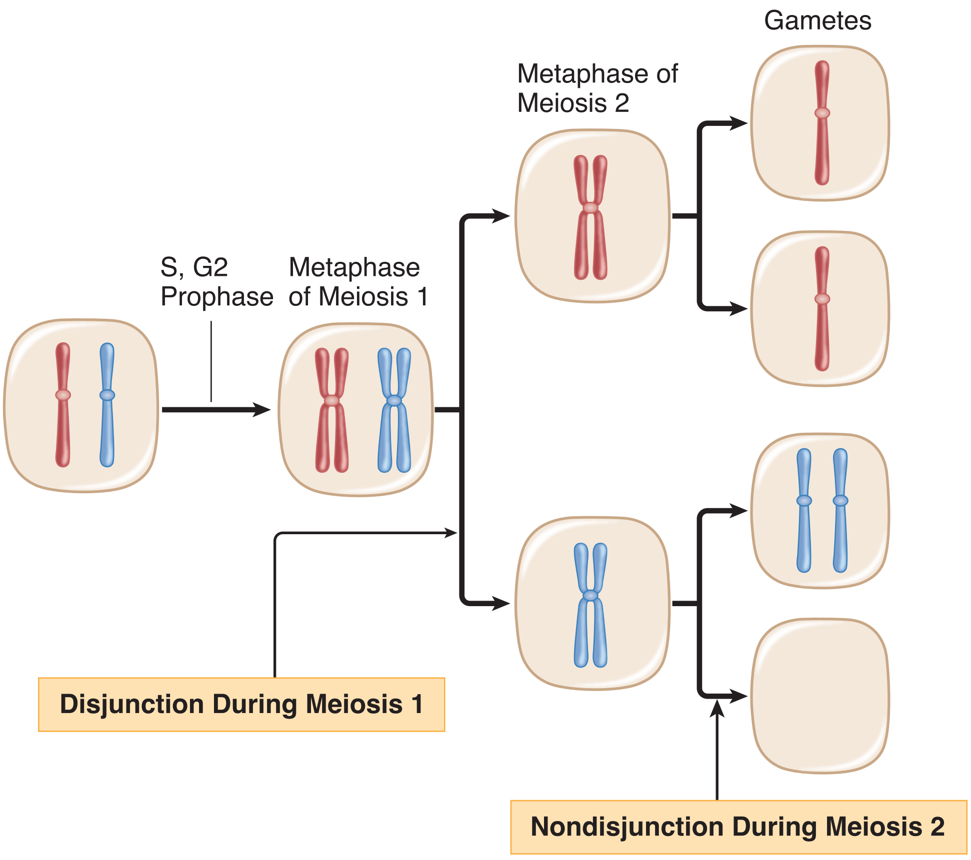


Figure II-3-2C. Nondisjunction During Meiosis 2

STRUCTURAL CHROMOSOME ABNORMALITIES

Structural alterations of chromosomes occur when chromosomes are broken by agents termed **clastogens** (e.g., radiation, some viruses, and some chemicals). Some alterations may result in a loss or gain of genetic material and are called **unbalanced** alterations; **balanced** alterations do not result in a gain or loss of genetic material and usually have fewer clinical consequences. As with other types of mutations, structural alterations can occur either in the germ line or in somatic cells. The former can be transmitted to offspring. The latter, although not transmitted to offspring, can alter genetic material such that the cell can give rise to cancer.

Translocations

High-Yield

Translocations occur when chromosomes are broken and the broken elements reattach to other chromosomes. Translocations can be classified into two major types: reciprocal and Robertsonian.

Reciprocal translocation

Reciprocal translocations occur when genetic material is exchanged between nonhomologous chromosomes; for example, chromosomes 2 and 8 (Figure II-3-3). If this happens during gametogenesis, the offspring will carry the reciprocal translocation in all his or her cells and will be called a **translocation carrier**. The karyotype would be 46,XY,t(2p;8p) or 46,XX,t(2p;8p). Because this individual has all of the genetic material (balanced, albeit some of it misplaced because of the translocation), there are often no clinical consequences other than during reproduction.

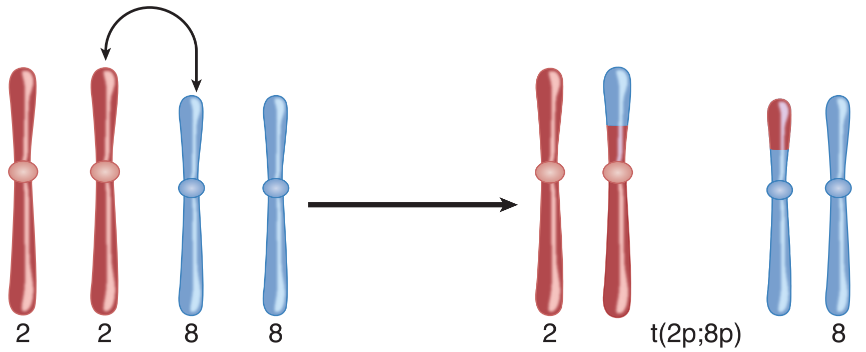


Figure II-3-3. A Reciprocal Translocation

Note

Alternate and **adjacent** segregation are diagrams (Figure II-3-4, upper right) used to predict the possible gametes produced by a translocation carrier.

- Adjacent segregation: chromosomes from adjacent quadrants (next to each other) enter a gamete
- Alternate segregation: chromosomes from alternate (diagonally opposed) quadrants enter a gamete

In a translocation carrier, during gametogenesis and meiosis, unbalanced genetic material can be transmitted to the offspring, causing partial trisomies and partial monosomies typically resulting in pregnancy loss. During meiosis 1, the translocated chromosomes may segregate as chromosome 8 or as chromosome 2, producing a variety of possible gametes with respect to these chromosomes.

For example, see Figure II-3-4, which depicts a man who is a translocation carrier mating with a normal woman. The diagram in the upper right is used to depict the possible sperm the father can produce. It acknowledges that the translocated chromosomes can potentially pair with either of the two homologs (2 or 8) during meiosis.

Sperm that contain balanced chromosomal material (labeled alternate segregation in the diagram) produce either a normal diploid conception or another translocation carrier. Both are likely to be live births.

Sperm that contain unbalanced chromosomal material (labeled adjacent segregation in the diagram) produce conceptions that have partial monosomies and partial trisomies. These conceptions are likely to result in pregnancy loss.

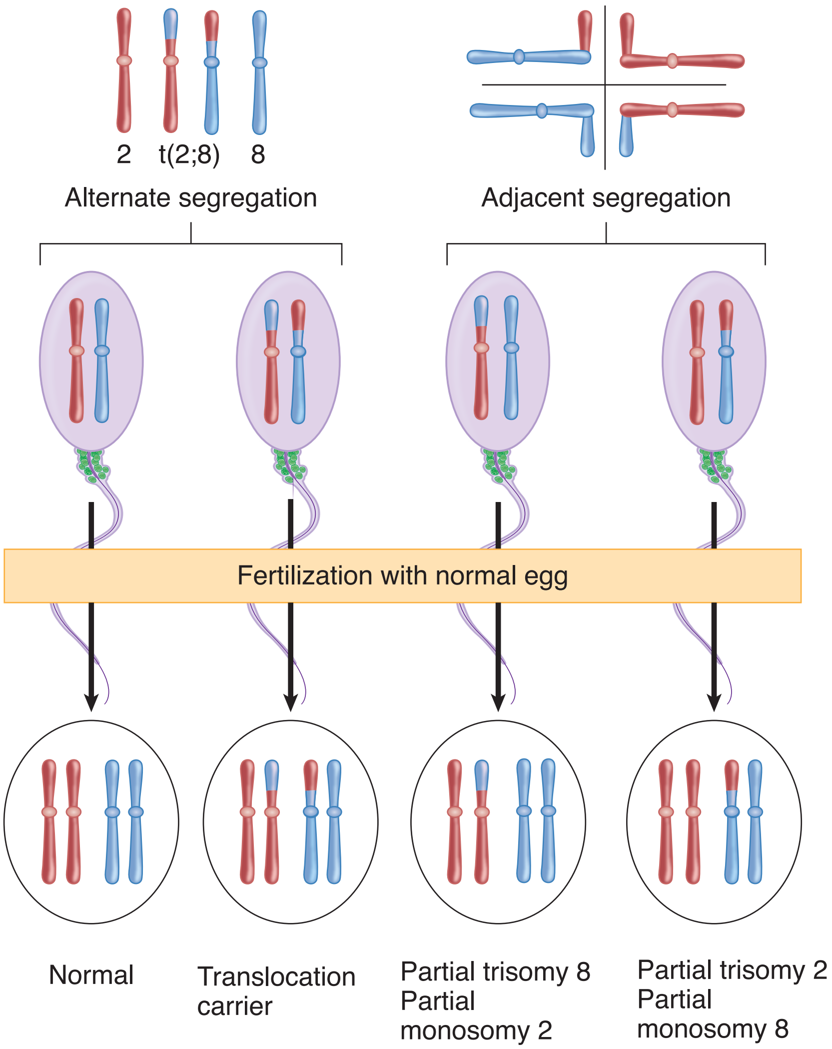


Figure II-3-4. Consequences of a Reciprocal Translocation (Illustrated with Male)

Reciprocal Translocations After Birth. Reciprocal translocations may occur by chance at the somatic cell level throughout life. Because these translocations involve only a single cell and the genetic material is balanced, there is often no consequence. Rarely, however, a reciprocal translocation may alter the expression or structure of an oncogene or a tumor suppressor gene, conferring an abnormal growth advantage to the cell.

Note

Reciprocal Translocations and Pregnancy Loss

When one parent is a reciprocal translocation carrier:

- Adjacent segregation produces unbalanced genetic material and most likely loss of pregnancy.
- Alternate segregation produces a normal haploid gamete (and diploid conception) or a liveborn who is a phenotypically normal translocation carrier.

Bridge to Pathology

Translocations Involving Oncogenes

Translocations are seen in a variety of cancers. Important examples include:

- t(9;22) chronic myelogenous leukemia (*c-abl*)
- t(15;17) acute myelogenous leukemia (*retinoid receptor-α*)
- t(14;18) follicular lymphomas (*bcl-2* that inhibits apoptosis)
- t(8;14) Burkitt lymphoma (*c-myc*)
- t(11;14) mantle cell lymphoma (cyclin D)

Chronic Myelogenous Leukemia and the Philadelphia Chromosome

Although most of our discussion deals with inherited chromosome alterations, rearrangements in somatic cells can lead to the formation of cancers by altering the genetic control of cellular proliferation. A classic example is a reciprocal translocation of the long arms of chromosomes 9 and 22, termed the *Philadelphia chromosome*. This translocation alters the activity of the *abl* proto-oncogene (proto-oncogenes can lead to cancer). When this alteration occurs in hematopoietic cells, it can result in chronic myelogenous leukemia. More than 100 different chromosome rearrangements involving nearly every chromosome have been observed in more than 40 types of cancer.

Robertsonian translocations

These translocations are much more common than reciprocal translocations and are estimated to occur in approximately 1 in 1,000 live births. They occur only in the acrocentric chromosomes (13, 14, 15, 21, and 22) and involve the loss of the short arms of two of the chromosomes and subsequent fusion of the long arms. An example of a Robertsonian translocation involving chromosomes 14 and 21 is shown in Figure II-3-5. The karyotype of this (male) translocation carrier is designated 45,XY,–14,–21,+t(14q;21q). Because the short arms of the acrocentric chromosomes contain no essential genetic material, their loss produces no clinical consequences, and the translocation carrier is not clinically affected.

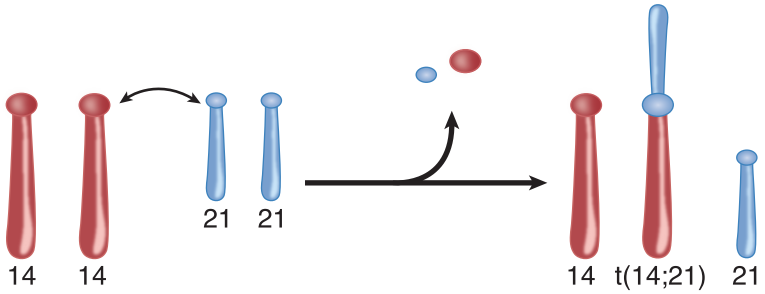


Figure II-3-5. A Robertsonian Translocation

When the carrier's germ cells are formed through meiosis, the translocated chromosome must pair with its homologs. If **alternate segregation** occurs, the offspring will inherit either a normal chromosome complement or will be a normal carrier like the parent. If **adjacent segregation** occurs, the offspring will have an unbalanced chromosome complement (an extra or missing copy of the long arm of chromosome 21 or 14). Because only the long arms of these chromosomes contain genetically important material, the effect is equivalent to a trisomy or monosomy.

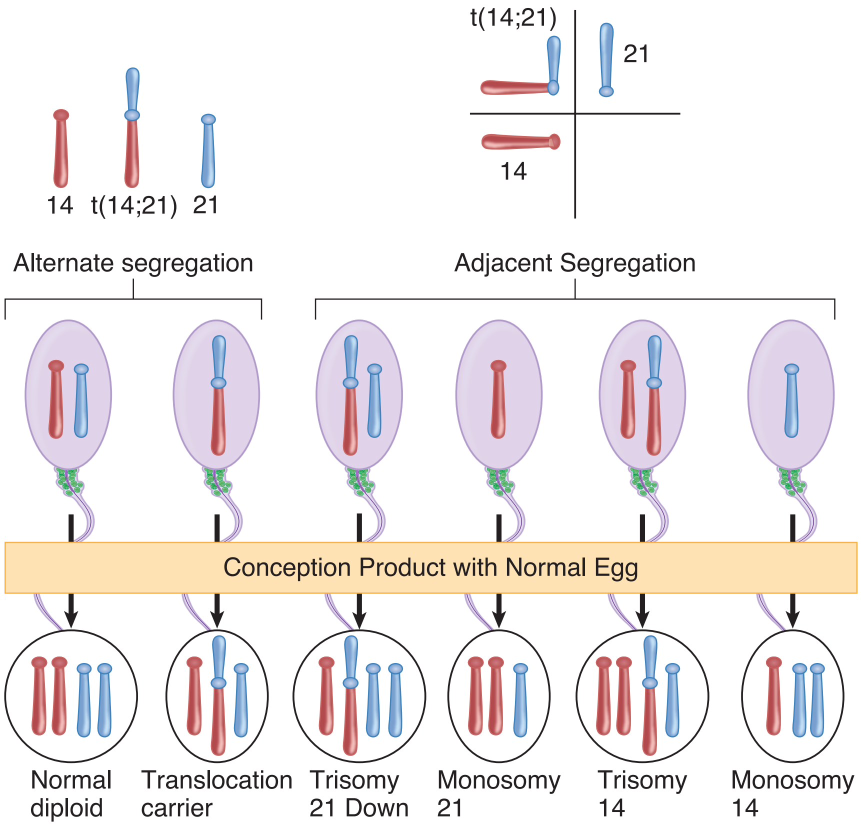


Figure II-3-6. Consequences of a Robertsonian Translocaton in One Parent (Illustrated with Male)

Robertsonian Translocation and Down Syndrome. Approximately 5% of Down syndrome cases are the result of a Robertsonian translocation affecting chromosome 14 and chromosome 21. When a translocation carrier produces gametes, the translocation chromosome can segregate with the normal 14 or with the normal 21. A diagram can be drawn to represent the 6 possible gametes that could be produced. Figure II-3-6 shows the diagram, the 6 sperm (in this example, the translocation carrier is a male), and the outcome of conception with a genetically normal woman.

Although adjacent segregation usually results in pregnancy loss, one important exception is that which produces trisomy 21. This may be a live birth, resulting in an infant with Down syndrome.

Note

Robertsonian Translocations

When one parent is a Robertsonian translocation carrier:

- Adjacent segregation produces unbalanced genetic material and most likely loss of pregnancy. Important exception: Down syndrome: 46,XX or 46XY,–14 +t(14q;21q)
- Alternate segregation produces a normal haploid gamete (and diploid conception) or a liveborn who is a phenotypically normal translocation carrier.

One can determine the mechanism leading to Down syndrome by examining the karyotype. Trisomy 21 due to nondisjunction during meiosis (95% of Down syndrome cases) has the karyotype 47,XX,+21 or 47,XY,+21. In the 5% of cases where Down syndrome is due to a Robertsonian translocation in a parent, the karyotype will be 46,XX,–14,+t(14q;21q) or 46,XY,–14,+t(14q;21q). The key difference is 47 versus 46 chromosomes in the individual with Down syndrome.

Although the recurrence risk for trisomy 21 due to nondisjunction during meiosis is very low, the recurrence risk for offspring of the Robertsonian translocation carrier parent is significantly higher. The recurrence risk (determined empirically) for female translocation carriers is 10–15%, and that for male translocation carriers is 1–2%.

The reason for the difference between males and females is not well understood. The elevated recurrence risk for translocation carriers versus noncarriers underscores the importance of ordering a chromosome study when Down syndrome is suspected in a newborn.

Down syndrome (nondisjunction during meiosis)	Down syndrome (parent carries a Robertsonian translocation)
• 47,XX,+21 or 47,XY.+21 • No association with prior pregnancy loss • Older mother • Very low recurrence rate	• 46,XX,–14,+t(14;21), or 46,XY,–14,+t(14;21) • May be associated with prior pregnancy loss • May be a younger mother • Recurrence rate 10–15% if mom is translocation carrier; 1–2% if dad is translocation carrier

Deletions

High-Yield

A deletion occurs when a chromosome loses some of its genetic information. Terminal deletions (the end of the chromosome is lost) and interstitial deletions (material within the chromosome is lost) may be caused by agents that cause chromosome breaks and by unequal crossover during meiosis.

Deletions can be large and microscopically visible in a stained preparation. The figure below shows both an interstitial deletion and a terminal deletion of 5p. Both result in Cri-du-chat syndrome.

- 46,XX or 46,XY, del(5p)
- High-pitched, cat-like cry
- Mental retardation, microcephaly
- Congenital heart disease

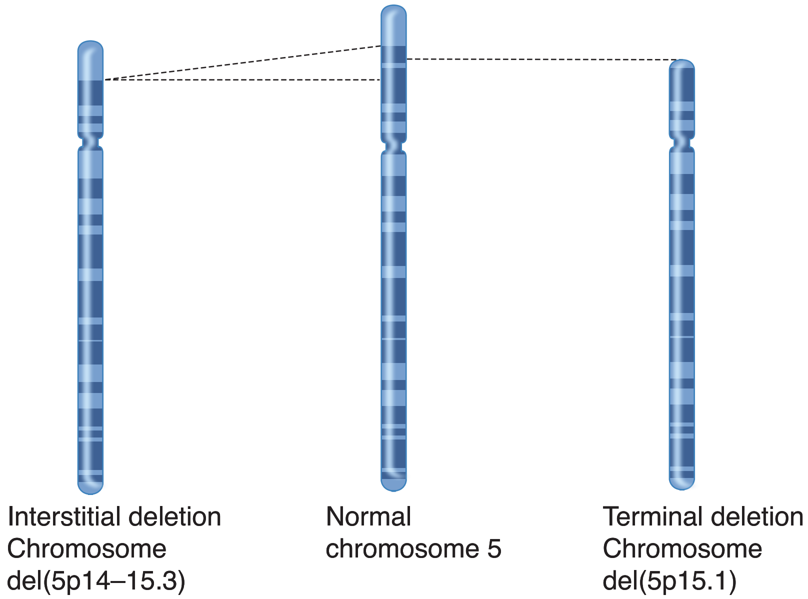


Figure II-3-7. Terminal and Interstitial Deletions of Chromosome 5p

Microdeletions

Some deletions may be so small that they are not readily apparent microscopically without special fluorescent probes (FISH). Examples include Prader-Willi and Angelman syndromes.

If a microdeletion includes several contiguous genes, a variety of phenotypic outcomes may be part of the genetic syndrome. Examples include:

- DiGeorge syndrome: congenital absence of the thymus and parathyroids, hypocalcemic tetany, T-cell immunodeficiency, characteristic facies with cleft palate, heart defects
- Wilms tumor: aniridia, genital abnormalities, mental retardation (WAGR)
- Williams syndrome: hypercalcemia, supravalvular aortic stenosis, mental retardation, characteristic facies

Note

Structural Abnormalities

- Translocations (Robertsonian and reciprocal)
- Deletions and duplications
- Inversions (pericentric and paracentric)
- Ring chromosomes
- Isochromosomes

OTHER CHROMOSOME ABNORMALITIES

Several other types of structural abnormalities are seen in human karyotypes. In general, their frequency and clinical consequences tend to be less severe than those of translocations and deletions.

Inversions

Inversions occur when the chromosome segment between two breaks is reinserted in the same location but in reverse order. Inversions that include the centromere are termed **pericentric**, whereas those that do not include the centromere are termed **paracentric**. The karyotype of the inversion shown in Figure II-3-8, extending from 3p21 to 3q13 is 46,XY,inv(3)(p21;q13). Inversion carriers still retain all of their genetic material, so they are usually unaffected (although an inversion may interrupt or otherwise affect a specific gene and thus cause disease). Because homologous chromosomes must line up during meiosis, inverted chromosomes will form loops that, through recombination, may result in a gamete that contains a deletion or a duplication, which may then be transmitted to the offspring.

Pericentric Inversion of Chromosome 16

A male infant, the product of a full-term pregnancy, was born with hypospadias and ambiguous genitalia. He had a poor sucking reflex, fed poorly, and had slow weight gain. He had wide-set eyes, a depressed nasal bridge, and microcephaly. The father stated that several members of his family, including his brother, had an abnormal chromosome 16. His brother had two children, both healthy, and the father assumed that he would also have normal children. Karyotype analysis confirmed that the father had a pericentric inversion of chromosome 16 and that his infant son had a duplication of material on 16q, causing a small partial trisomy.

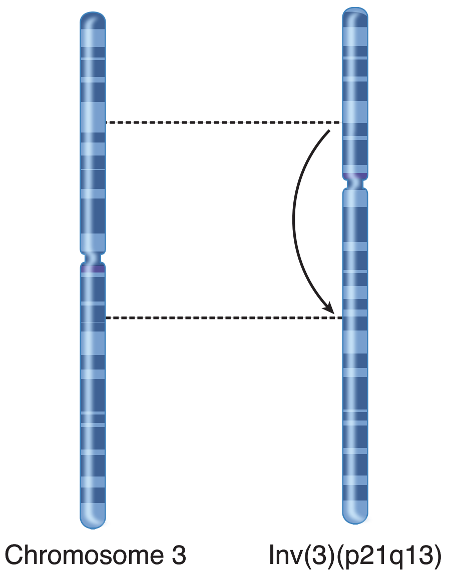


Figure II-3-8. A Pericentric Inversion of Chromosome 3

Ring Chromosome

A ring chromosome can form when a deletion occurs on both tips of a chromosome and the remaining chromosome ends fuse together. The karyotype for a female with a ring chromosome X would be 46,X,r(X). Ring chromosomes are often lost, resulting in a monosomy (e.g., loss of a ring X chromosome would produce Turner syndrome). These chromosomes have been observed at least once for each human chromosome.

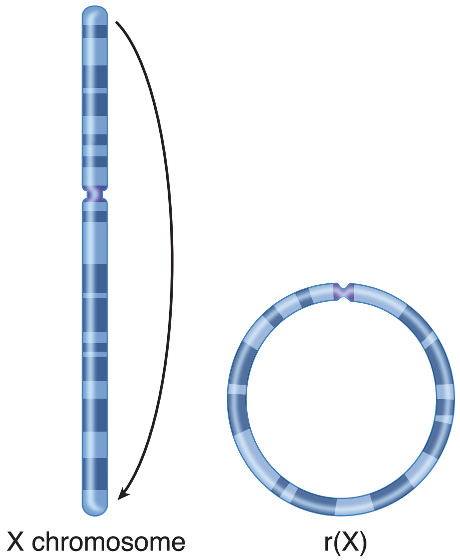


Figure II-3-9. Ring X-Chromosome

Isochromosome

High-Yield

When a chromosome divides along the axis perpendicular to its normal axis of division, an **isochromosome** is created (i.e., 2 copies of one arm but no copy of the other). Because of the lethality of autosomal isochromosomes, most isochromosomes that have been observed in live births involve the X chromosome, as shown in Figure II-3-10. The karyotype of an isochromosome for the long arm of the X chromosome would be 46,X,i(Xq); this karyotype results in an individual with Turner syndrome, indicating that most of the critical genes responsible for the Turner phenotype are on Xp.

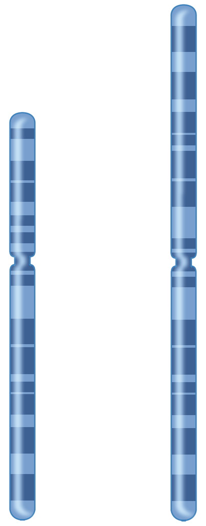


Figure II-3-10. Isochromosome Xq

ADVANCES IN MOLECULAR CYTOGENETICS

Although chromosome abnormalities are still commonly visualized by examining metaphase chromosomes under a microscope, several powerful new techniques combine cytogenetics with modern molecular methods. Two of the most important techniques are described here.

Fluorescence *in situ* Hybridization (FISH)

High-Yield

In fluorescence *in situ* hybridization (FISH), a chromosome-specific DNA segment is labeled with a fluorescent tag to create a **probe**. This probe is then hybridized with the patient's chromosomes, which are visualized under a fluorescence microscope.

Because the probe will hybridize only with a complementary DNA sequence, the probe will mark the presence of the chromosome segment being tested. For example, a probe that is specific for chromosome 21 will hybridize in 3 places in the cells of a trisomy 21 patient, providing a diagnosis of Down syndrome.

FISH is also commonly used to detect deletions. An analysis using a probe that hybridizes to the region of 15q corresponding to Prader-Willi syndrome (*see* Chapter 1) will show only a single signal in a patient, confirming the diagnosis of this deletion syndrome.

An advantage of FISH is that chromosomes do not have to be in the metaphase stage for accurate diagnosis. Even though interphase and prophase chromosomes cannot be clearly visualized themselves, the number of hybridization signals can still be counted accurately.

Note

FISH analysis detects aneuploidies, translocations, and deletions (including microdeletions).

Spectral Karyotyping

Spectral karyotyping involves the use of 5 different fluorescent probes that hybridize differentially to different sets of chromosomes. In combination with a special camera and image-processing software, this technique produces a karyotype in which every chromosome is "painted" a different color. This allows for the ready visualization of chromosome rearrangements such as small translocations, e.g., the Philadelphia chromosome rearrangement t(9;22) involved in chronic myelogenous leukemia.

Review Questions

Select the ONE best answer.

1. A 26-year-old woman has produced two children with Down syndrome, and she has also had two miscarriages. Which of the following would be the best explanation?

   A. Her first cousin has Down syndrome.

   B. Her husband is 62 years old.

   C. She carries a reciprocal translocation involving chromosomes 14 and 18.

   D. She carries a Robertsonian translocation involving chromosomes 14 and 21.

   E. She was exposed to multiple x-rays as a child.

2. A 6-year-old boy has a family history of mental retardation and has developmental delay and some unusual facial features. He is being evaluated for possible fragile X syndrome. Which of the following would be most useful in helping establish the diagnosis?

   A. Genetic test for a trinucleotide repeat expansion in the fragile X gene

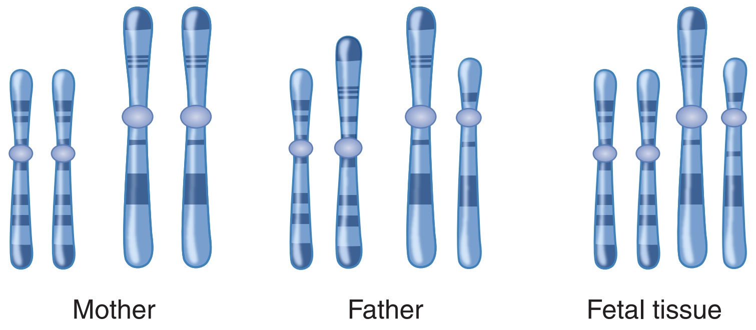
   B. IQ test

   C. Karyotype of the child's chromosomes

   D. Karyotype of the father's chromosomes

   E. Measurement of testicular volume

3. A couple has one son, who is age 7. Multiple attempts to have a second child have ended in miscarriages and spontaneous abortions. Karyotypes of the mother, the father, and the most recently aborted fetus are represented schematically below. What is the most likely explanation for the most recent pregnancy loss?



   A. Aneuploidy in the fetus

   B. Fetus identified as a reciprocal translocation carrier

   C. Nondisjunction during oogenesis in the mother

   D. Partial monosomy and trisomy in the fetus

   E. Unbalanced chromosomal material in the father

4. A woman brings her 16-year-old daughter to a physician because she has not yet begun menstruating. Although her parents are both 1.75 meters, the patient is 1.5 meters and has always been below the 50th percentile in height. Physical examination reveals no breast development. She has no problems in school and is of normal intelligence. What is the most likely underlying basis for her condition?

   A. A 45,X karyotype
   B. A balanced reciprocal translocation
   C. A balanced Robertsonian translocation
   D. Two Barr bodies
   E. Deletion of an imprinted locus

5. A 38-year-old woman in her 15th week of pregnancy undergoes ultrasonography that reveals an increased area of nuchal transparency. Amniocentesis is recommended and performed at 16 weeks' gestation. The amniotic karyotype is 46,XYadd(18)(p.11.2), indicating additional chromosomal material on the short arm of one chromosome 18 at band 11.2. All other chromosomes are normal. What is the most likely cause of this fetal karyotype?

   A. A balanced reciprocal translocation in one of the parents
   B. A balanced Robertsonian translocation in one of the parents
   C. An isochromosome 18i(p) in one of the parents
   D. Nondisjunction during meiosis 1 in one of the parents
   E. Nondisjunction during meiosis 2 in one of the parents

6. A 37-year-old woman is brought to emergency department because of crampy abdominal pain and vaginal bleeding for 3 hours. She is 11 weeks pregnant. This is her first pregnancy. Her pregnancy has been unremarkable until this episode. Her temperature is 36.8 C (98.2 F), pulse is 106/min, blood pressure is 125/70 mm Hg, and respiration rate is 22/min. Speculum examination shows the presence of blood in the vagina and cervical dilatation. Inevitable spontaneous abortion is suspected. After discussing the condition with the patient, she gave her consent for dilatation and curettage. What is the most common cause of spontaneous abortions?

   A. Chromosomal abnormality, polyploidy
   B. Chromosomal abnormality, monosomy X
   C. Chromosomal abnormality, trisomy
   D. Effects of environmental chemicals
   E. Immunologic rejection
   F. Infection
   G. Maternal endocrinopathies
   H. Physical stresses
   I. Teratogenic drugs

Answers

1. **Answer: D.** As a translocation carrier, it is possible that she can transmit the translocated chromosome, containing the long arms of both 14 and 21, to each of her offspring. If she also transmits her normal copy of chromosome 21, then she will effectively transmit two copies of chromosome 21. When this egg cell is fertilized by a sperm cell carrying another copy of chromosome 21, the zygote will receive 3 copies of the long arm of chromosome 21. The miscarriages may represent fetuses that inherited 3 copies of the long arm and were spontaneously aborted during pregnancy.

   Although the risk for Down syndrome increases if a woman has had a previous child, there is no evidence that the risk increases if a more distant relative, such as a first cousin, is affected **(choice A)**.

   Although there is no conclusive evidence for an increased risk of Down syndrome with advanced maternal age, there is little or no evidence for a paternal age effect on Down syndrome risk **(choice B)**.

   An extra copy of material from chromosome 14 or 18 **(choice C)**could result in a miscarriage, but neither would produce children with Down syndrome, which is caused by an extra copy of the long arm of chromosome 21.

   Heavy irradiation has been shown to induce nondisjunction in some experimental animals, but there is no good evidence for a detectable effect on human trisomy **(choice E)**.

2. **Answer: A.** The presence of an expanded trinucleotide repeat in the 5′ untranslated region of the gene is an accurate test for fragile X syndrome.

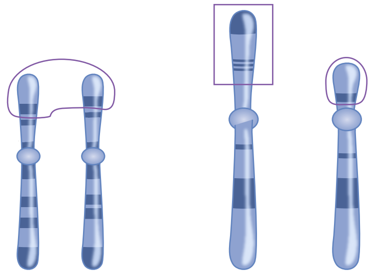
   An IQ test **(choice B)**would be useful because the IQ is typically much lower than average in fragile X syndrome patients. However, many other syndromes also include mental retardation as a feature, so this would not be a specific test.

   A karyotype of the child’s chromosomes **(choice C)** might reveal X chromosomes with the decondensed long arm characteristic of this syndrome, but not all chromosomes have this appearance in affected individuals. Thus, the karyotype may yield a false-negative diagnosis.

   The father’s chromosomes **(choice D)** will not be relevant because this is an X-linked disorder.

   Testicular volume **(choice E)** is increased in males with fragile X syndrome, but this is observed in postpubertal males.

3. **Answer: D.** The fetal karyotype shows a partial trisomy and a partial monosomy.



Fetal tissue

The fetus has 46 chromosomes, indicating euploidy (a multiple of 23), not aneuploidy **(choice A)**. The father is the reciprocal translocation carrier, not the fetus **(choice B)**. Nondisjunction during meiosis **(choice C)** produces full trisomies and chromosomes have normal structure. Although the father is a translocation carrier, his genetic material is balanced, not unbalanced **(choice E)**. He is diploid for all loci.

4. **Answer: A.** The daughter most likely has Turner syndrome, monosomy X, or 45,X. It should be high on the differential diagnosis list for a female adolescent of short stature who presents with primary amenorrhea. Women with Turner syndrome have streak gonads, and the absence of ovarian function is responsible for failure to develop many secondary sex characteristics.

   Balanced translocations **(choices B and C)** have few, if any, consequences on the phenotype, although they may result in pregnancy loss of conceptions with unbalanced chromosome material.

   Women with Turner syndrome have no Barr body. Two Barr bodies **(choice D)** would be consistent with a 47,XXX karyotype or a 48,XXXY karyotype, neither of which produces the phenotype described.

   Deletion of a locus subject to imprinting **(choice E)** is consistent with Prader-Will syndrome or Angelman syndrome but is not associated with the phenotype described.

5. **Answer: A.** The fetus has unbalanced chromosomal material (additional chromosomal material on one copy of chromosome 18). One of the parents is likely to be a carrier of a reciprocal translocation involving chromosome 18 and one other chromosome (unspecified in stem).

   A Robertsonian translocation **(choice B)** would result in fusion of q arms from two acrocentric chromosomes. This is not what is described in the fetal karyotype.

   Isochromosome 18(p) indicates a chromosome 18 with two p arms and no q arms **(choice C)**. This is not the abnormality described in the fetal karyotype.

   Nondisjunction during either meiosis 1 or meiosis 2 **(choices D and E)** would produce a full trisomy.

6. **Answer: C.** Chromosomal abnormalities are responsible for about 50% of first trimester spontaneous abortions, and of these the most common cause is trisomy (52%). The most common trisomy in spontaneous abortion is trisomy 16. Polyploidy (**choice A**) is seen in 22% and monosomy **(choice B)** in 19%.

   All other listed causes can also cause miscarriage; however, these problems are less common than chromosomal anomalies.

Genetics of Common Diseases 4

Learning Objectives

- ❑ Interpret scenarios about multifactorial inheritance

OVERVIEW

Previous discussion has dealt with diseases caused by an alteration in a single gene or in a specific chromosome. Most common diseases (heart disease, cancer, diabetes, etc.) have substantial genetic components, but their causation is complex. These diseases tend to cluster in families (familial), but they do not conform to mendelian pedigree patterns. This chapter reviews some basic principles of the genetics of common, complex diseases.

MULTIFACTORIAL INHERITANCE

The term *multifactorial inheritance* refers to the fact that most common diseases are caused by multiple genes (i.e., a polygenic component) and expression is often influenced by environmental factors. The genetic and environmental factors involved are referred to as **risk factors,** and the summation of these in an individual represents that person's **liability** for the disease. Because several genes and influential environmental factors contribute to the liability, its distribution in the population can be represented as a Gaussian ("bell-shaped") curve. Figure II-4-1A shows an example of a distribution curve for body mass index (BMI), a multifactorial trait, in the U.S. adult population. The mean BMI is 26 ± 6 kg/m^2. Because obesity is defined in terms of BMI (BMI $\geq$30 kg/m^2), this graph can also represent a liability curve for obesity.

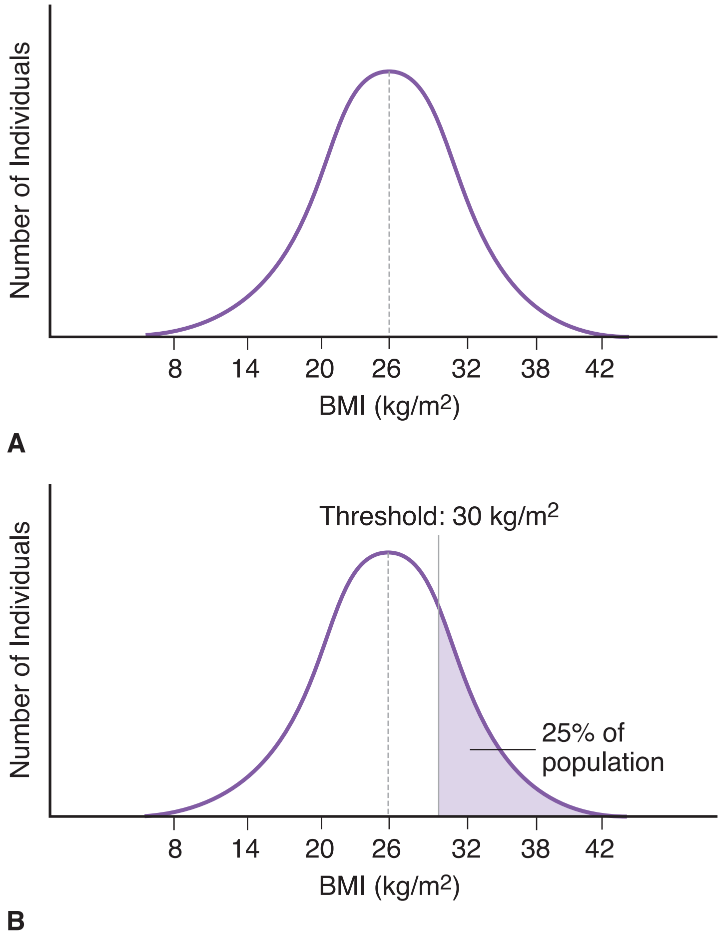


Figure II-4-1. Obesity in the U.S. Population.
(A) Distribution of BMI in the U.S. Population
(B) Threshold for and Prevalence of Obesity

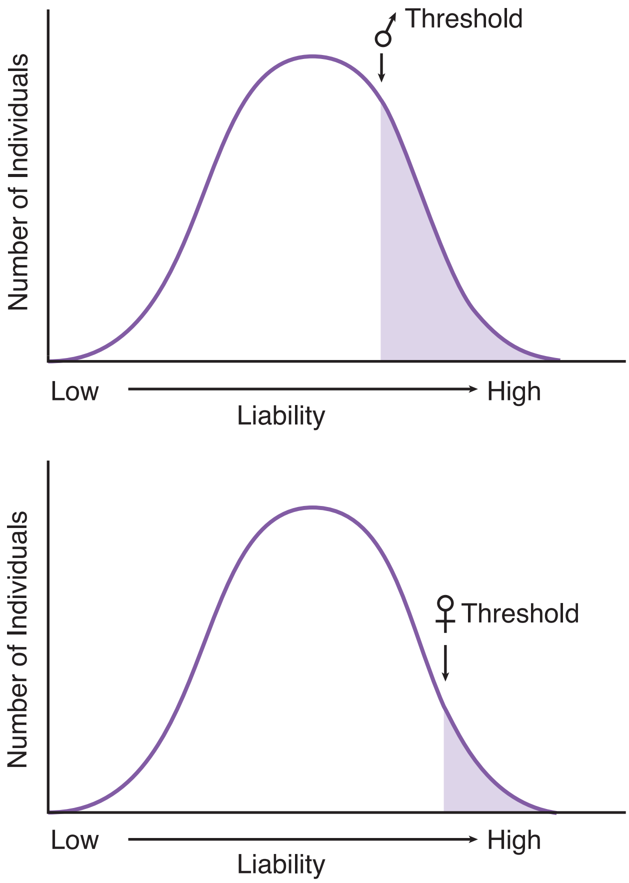
Multifactorial Threshold Model

Unlike liability for a disease, the multifactorial diseases themselves are not continuous traits. Either a person has the disease or does not; i.e., it is a discontinuous characteristic. Expression of the disease phenotype occurs only when a certain threshold of liability is reached. The threshold for a complex disease is set by the diagnostic criteria. As a simple example, obesity is a complex, multifactorial condition in which excess body fat may put a person at risk for a variety of other conditions, including type 2 diabetes and cardiovascular disease (see below). The clinical definition of obesity is a BMI $\geq$30 kg/m^2. If one plots BMI for the population of the United States and sets the "obesity threshold" at 30 kg/m^2, nearly 25% of adults over 20 years of age will be above this threshold. Thus, the prevalence of obesity in this population is 25% (0.25; 1 in 4).

For some common diseases, the male and female thresholds are different (Figure II-4-2.) For example, risk factors for atherosclerosis and myocardial infarction include:

- LDL-receptor deficiency
- Hyperlipidemia
- Smoking
- Diabetes
- Obesity
- Lack of exercise
- Elevated homocysteine
- Male sex

Some of these risk factors are more completely genetic (LDL-receptor deficiency), some are more completely environmental (lack of exercise and smoking), and some have substantial genetic and environmental contributions (obesity). The contributing factors and an individual's liability are usually determined empirically, as are the recurrence risks.



The male threshold is lower than the female threshold, so the prevalence of the disease is higher in males than in females.

Figure II-4-2. Multifactorial Diseases May Have Different Male and Female Thresholds

Assessing Recurrence Risks for Multifactorial Diseases

The inheritance patterns of multifactorial diseases differ from those of single-gene disorders in several important ways. Recurrence risks:

- **Are estimated empirically.** For single-gene disorders, the mechanism of gene action is understood (e.g., cystic fibrosis is caused by an autosomal recessive mutation, neurofibromatosis is produced by an autosomal dominant mutation, etc.), and recurrence risks can be derived based on known principles of inheritance. In contrast, the genes and environmental factors underlying multifactorial traits have not been identified specifically. Consequently, empirical recurrence risks (i.e., based on direct observation of data) must be derived. For example, if we wish to know the recurrence risk for siblings of individuals with cleft lip and/or palate, we ascertain a large cohort of individuals with cleft lip and/or palate and then measure the proportion of their siblings who are also affected with cleft lip and/or palate (in this case, the sibling recurrence risk is approximately 3%, which is considerably higher than the general population prevalence of 0.1%).
- **Increase as the number of affected relatives increases.** Recurrence risks for single-gene traits remain the same regardless of the number of affected individuals in the family (e.g., the recurrence risk for cystic fibrosis is 25% in a carrier-by-carrier mating, even if several previous siblings have all been affected). Multifactorial recurrence risks increase as the number of affected relatives (e.g., siblings) increases. This does not mean that the true risk has changed; rather, it reflects the fact that additional affected individuals provide more information about the true risk. The presence of multiple affected individuals indicates that the family is located higher on the liability distribution (i.e., they likely have more genetic and environmental risk factors). For example, one study showed that sibling recurrence risk for a neural tube defect (spina bifida or anencephaly; *see* Clinical Correlate) was 3% if one sibling was affected, 12% if two were affected, and 25% if 3 were affected.
- **Increase as the severity of the disease expression increases.** Again, this reflects the fact that the individual and his or her relatives are located higher on the liability distribution.
- **Increase if the affected individual (i.e., the proband) is a member of the less commonly affected sex.** This principle follows from the fact that an affected individual of the less commonly affected sex will be, on average, higher on the liability distribution (Figure II-4-2). For example, the prevalence of pyloric stenosis (congenital constriction of the pylorus) is approximately 1/1,000 for females and 1/200 for males. Thus, the average affected female is likely to be located higher on the liability distribution than is an affected male (i.e., the female has more genetic and environmental risk factors). The presence of more risk factors implies that the affected female's relatives are more likely to be affected than are the affected male's relatives.
- **Decrease rapidly for more remotely related relatives.** For example, one study of autism reported a sibling risk of 4.5%, an uncle–niece risk of 0.1%, and a first-cousin risk of 0.05%. In contrast, the risk of carrying a single-gene mutation decreases by only 1/2 with each

Note

Recurrence Risks for Multifactorial Diseases

- Are estimated empirically
- Increase as the number of affected relatives increases
- Increase as the severity of the disease expression increases
- Increase if the affected individual is a member of the less commonly affected sex
- Decrease very rapidly for more remotely related relatives
- Increase as the prevalence of the disease increases in a population

successive degree of relationship (i.e., 50% chance for siblings, 25% for uncle–niece relationships, and 12.5% for first cousins).

- **Increase as the prevalence of the disease increases in a population.** Although the recurrence risk for a single-gene disorder remains the same regardless of the prevalence of the disease in a population, the empirical risk for multifactorial diseases increases as the population prevalence increases. This is because populations with higher prevalence rates have a higher preponderance of genetic and environmental risk factors. This in turn raises the risk for relatives of affected individuals.

Clinical Correlate: Neural Tube Defects

Neural tube defects (NTDs) are one of the most common congenital malformations and are seen in approximately 1 in 1,000 births in the United States. **Anencephaly** (partial or complete absence of the brain) usually leads to a stillbirth, and anencephalics that survive to term do not live for more than a few days. **Spina bifida**, a protrusion of spinal tissue through the vertebral column, produces secondary hydrocephalus in 75% of cases and often produces some degree of paralysis. Improved intervention strategies have increased survival rates substantially for this condition, with more than two thirds of patients now surviving beyond 10 years of age. The sibling recurrence risk for NTDs is estimated to be 2–5%, which is much higher than the population prevalence. Thus, the disorder clusters in families. Recent epidemiologic studies show that 50–70% of NTDs can be prevented by periconceptional dietary folic acid supplementation. Folic acid deficiency is likely to be present in successive pregnancies, providing a nongenetic explanation for some of the familial clustering of this disease. However, there is also evidence for genetic variation in the ability to metabolize folic acid. Thus, NTDs provide an example in which familial clustering is likely related to both genetic and nongenetic factors.

Note

The exam expects you to know that pregnant women should take 400 μg of folic acid per day to prevent NTDs.

Genetics of Common Diseases: Summary of Principles

Several key principles should emerge from this review of the genetics of common diseases:

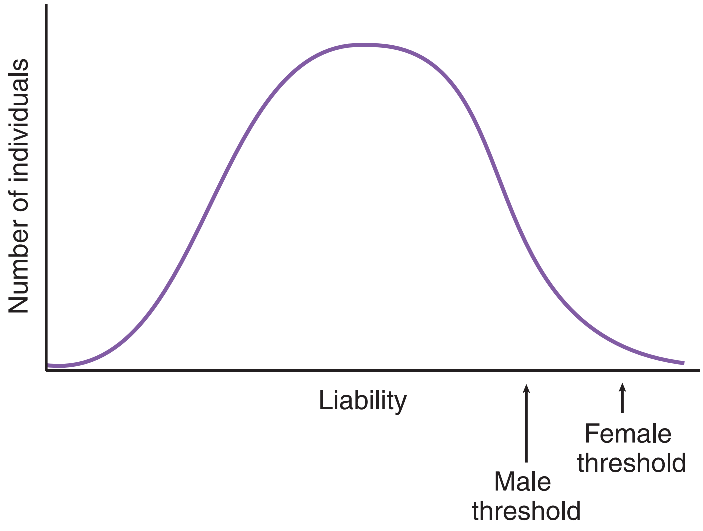
- Common diseases generally have both genetic and environmental liability factors.
- Liability for common diseases in a population can be represented by a normal (Gaussian) distribution.
- The disease threshold is set by diagnostic criteria and may be different for males and females. The fraction (or percent) of the population above the threshold defines the prevalence of the disease in that population.
- Recurrence risks within a family are determined empirically. Recurrence risks increase with the number of affected relatives, the severity of disease expression in the family, the probands of the less commonly affected sex, and the prevalence of disease in the population. Recurrence risks decrease rapidly for remotely related relatives.

- Heritability represents the contribution of genes to the liability curve. Heritability can be estimated by twin studies and adoption studies.
- For many common diseases, subsets of cases exist in which genetic factors play an especially important role. These subsets tend to develop disease early in life (e.g., *BRCA1* and *BRCA2* mutations in breast cancer), and they often tend to have a more severe expression of the disease.
- In some cases, genes involved in the less common, strongly inherited subsets of common diseases (in cancer, specific oncogenes and tumor suppressor genes) are also involved in the common noninherited cases (but in different ways, such as a somatic mutation instead of a germ-line mutation).

Review Questions

Select the ONE best answer.

1. Pyloric stenosis is 5 times more common in males than in females in certain Japanese populations. The liability curve for the development of this condition in that population is shown below:



Within this population, which of the following is most at risk for the development of disease?

A. The daughters of affected fathers

B. The daughters of affected mothers

C. The sons of affected fathers

D. The sons of affected mothers

Answers

1. **Answer: D.** Because the trait in this case is 5 times more common in males in females, it means that males are found lower on the liability curve. Fewer factors are needed to cause the disease phenotype in the male. Therefore, a female with the disease is higher on the liability curve and has a larger number of factors promoting disease. The highest risk population in this model of multifactorial inheritance would be the sons (the higher risk group) of affected mothers (the lower risk group). The affected mother had an accumulation of more disease-promoting liabilities, so she is likely to transmit these to her sons, who need fewer liabilities to develop the syndrome.

Recombination Frequency 5

Learning Objectives

- ❏ Solve problems concerning polymorphic markers and linkage analysis
- ❏ Demonstrate understanding of determining recombination frequency accurately
- ❏ Explain information related to LOD scores

OVERVIEW

An important step in understanding the basis of an inherited disease is to locate the gene(s) responsible for the disease. This chapter provides an overview of the techniques that have been used to map and clone thousands of human genes.

POLYMORPHIC MARKERS AND LINKAGE ANALYSIS

A prerequisite for successful linkage analysis is the availability of a large number of highly polymorphic markers dispersed throughout the genome. There are several classes of polymorphic markers; over 20,000 individual examples of these polymorphic markers at known locations have been identified and are available for linkage studies. Collectively, these markers provide a marker map of each chromosome, so when one wishes to map the position of a gene—often one involved in a disease process—the gene's position can be located relative to one or more markers by recombination mapping. These high-density marker maps allow genome-wide screening for mapping genes.

Note

Chromosome gene and marker maps are available online at pubmed.gov.

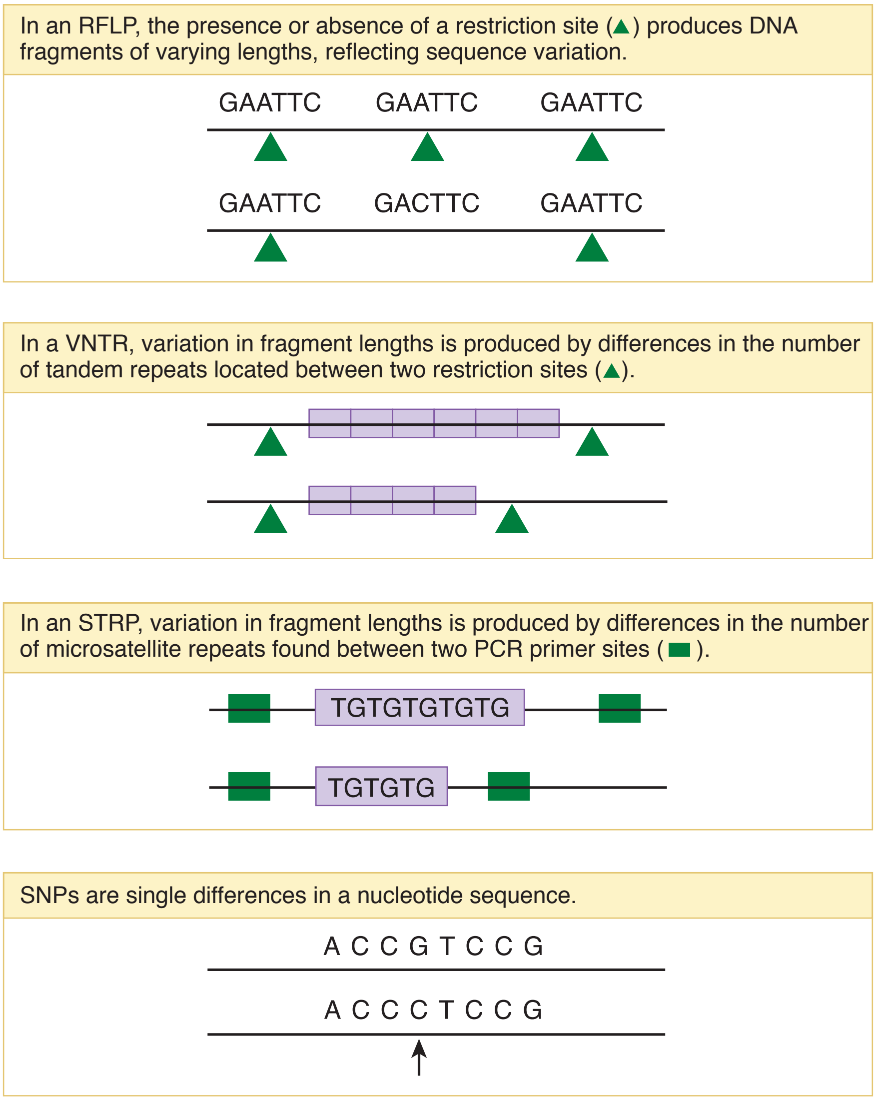


Figure II-5-1. Different Types of DNA Polymorphisms

RFLPs (restriction fragment length polymorphisms)

Restriction endonuclease (RE) sites—palindromes recognized by specific restriction endonucleases—can serve as 2-allele markers. A specific site may be present in some individuals (allele 1) and absent in others (allele 2), producing different-sized restriction fragments that can be visualized on a Southern blot.

Alternatively, the area containing the restriction site can be amplified by using a PCR (polymerase chain reaction) and treating the PCR product with the restriction enzyme to determine whether one (RE site absent) or two (RE site present) fragments are produced. The PCR product(s) can be separated by agarose gel electrophoresis and visualized directly without using a blot.

VNTRs (variable number of tandem repeats)

These polymorphisms are the result of varying numbers of minisatellite repeats in a specific region of a chromosome. The minisatellite repeat units typically range in size from 20 to 70 bases each. The repeat is flanked on both sides by a restriction site, and variation in the number of repeats produces restriction fragments of varying size. VNTRs are used infrequently in current genetic mapping, as they tend to cluster near the ends of chromosomes.

STRPs (short tandem repeat polymorphisms, or microsatellites)

Short tandem repeat polymorphisms are repetitive sequences in which the repeated unit is generally 2–6 bases long. An example of a dinucleotide repeat (CA/TG) is shown above. These markers have many alleles in the population, with each different repeat length at a locus representing a different allele. STRPs can be amplified with a PCR by using primers designed to flank the repeat block. Variation in the number of repeats produces PCR products of varying length, which can then be visualized on agarose gel electrophoresis. STRPs are distributed throughout the chromosomes, making them very useful in mapping genes. STRPs are used in paternity testing and in forensic cases, but these sequences can also be used in gene mapping.

SNPs (single nucleotide polymorphisms)

SNPs represent nucleotide positions in the human genome where only two nucleotides—for example, C or G—are found. These occur on average about once in every 1,000 base pairs (bp) and, like STRPs, are very useful in mapping genes. Unlike STRPs, which have multiple alleles at each locus, SNPs are usually two-allele markers (either G or C in the above example). These can be typed by PCR amplification and identification by sequencing or through the use of probes on DNA chips, a process which can be automated.

GENE MAPPING: LINKAGE ANALYSIS

Crossing Over, Recombination, and Linkage

The first step in gene mapping is to establish linkage with a known polymorphic marker (one with at least two alleles in the population). This can be done by recombination mapping to determine whether the gene is near a particular marker. Multiple markers on different chromosomes are used to establish linkage (or the lack of it).

Recombination mapping is based on crossing over during meiosis, the type of cell division that produces haploid ova and sperm. During prophase I of meiosis, homologous chromosomes line up and occasionally exchange portions of their DNA. This process (shown in Figure II-5-2) is termed **crossover**.

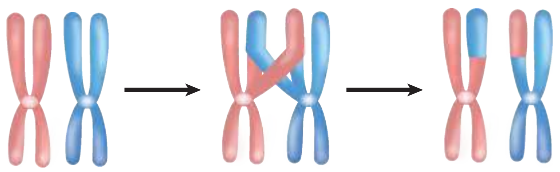


Figure II-5-2. The Process of Crossing Over Between Homologous Chromosomes

When a crossover event occurs between two loci, G and M, the resulting chromosomes **may** contain a new combination of alleles at loci G and M. When a new combination occurs, the crossover has produced a **recombination**. Because crossover events occur more or less randomly across chromosomes, loci that are located farther apart are more likely to experience an intervening crossover and thus a recombination of alleles. Recombination frequency provides a means of assessing the distance between loci on chromosomes, a key goal of gene mapping.

This process is illustrated in Figure II-5-3.

- If the gene of interest (with alleles G_1 and G_2) and the marker (with alleles M_1 and M_2) are on different chromosomes, the alleles will remain together in an egg or a sperm only about 50% of the time. They are **unlinked**.
- If the gene and the marker are on the same chromosome but are far apart, the alleles will remain together about 50% of the time. The larger distance between the gene and the marker allows multiple crossovers to occur between the alleles during prophase I of meiosis. An odd number of crossovers separates G_1 from M_1 (recombination), whereas an even number of crossovers places the alleles together on the same chromosome (no recombination). The gene and marker are again defined as **unlinked**.
- If the gene and the marker are close together on the same chromosome, a crossover between the two alleles is much less likely to occur. Therefore, G_1 and M_1 are likely to remain on the same chromosome more than 50% of the time. In other words, they show **less than 50% recombination**. The gene and the marker are now defined as **linked**.

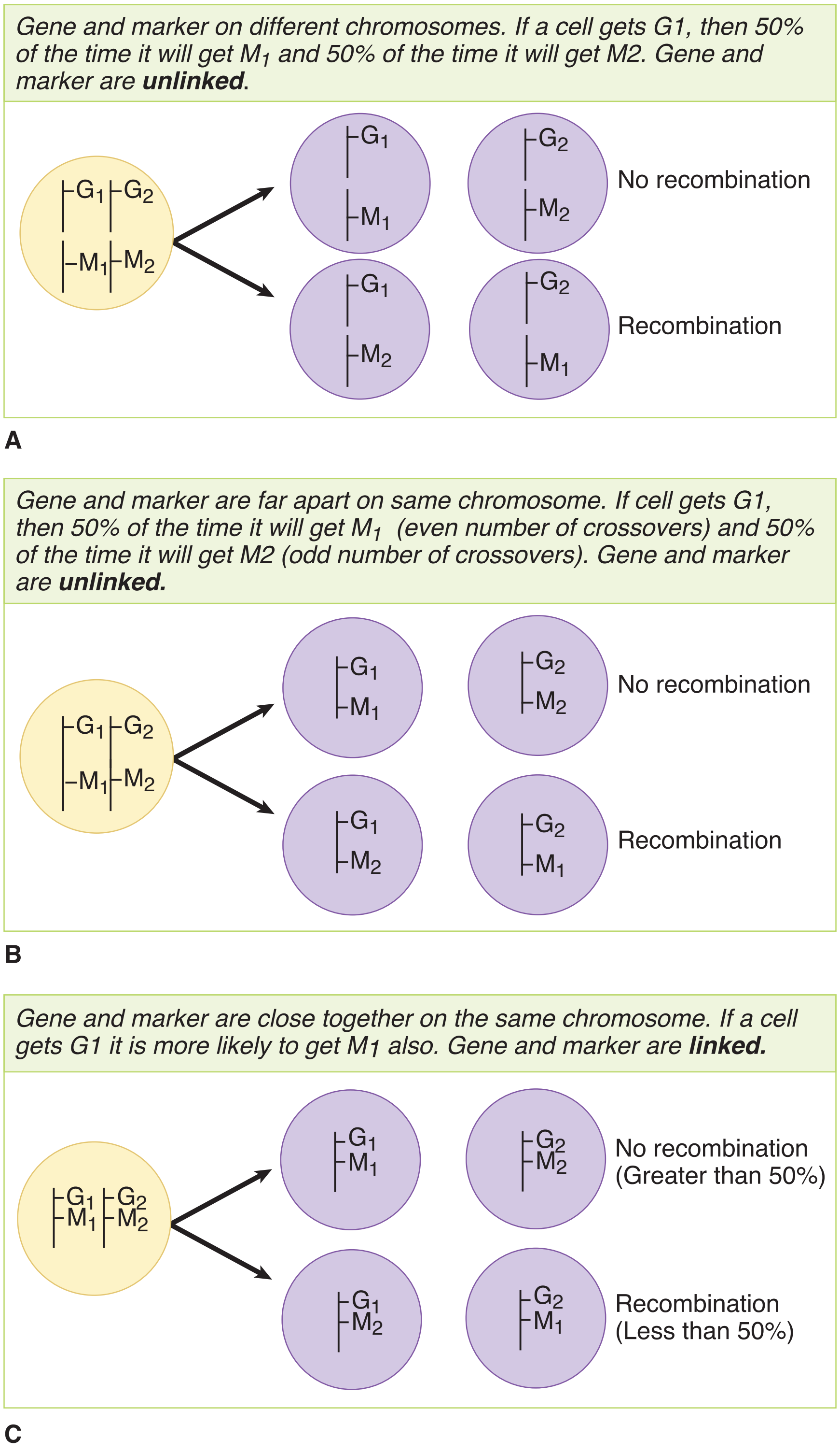


Figure II-5-3. Gene Mapping by Recombination Frequency with a Marker Whose Locus Is Known

Note

- G1 and G2 are alleles of a gene to be mapped.
- M1 and M2 are alleles of a marker whose locus is known.

Recombination Frequencies and Gene Mapping

High-Yield

The closer together two linked loci are—for instance, a gene and a marker—the lower the recombination frequency will be between them. Therefore, recombination frequency can be used to estimate proximity between a gene and a linked marker.

The following example of a family with neurofibromatosis type 1, an autosomal dominant disorder with complete penetrance, illustrates the concept of recombination frequency. Some members of the family have the disease-producing allele of the gene (indicated by phenotype in the pedigree) whose location is to be determined. Other individuals have the normal allele of the gene.

Each individual has also been typed for his or her allele(s) of a 2-allele marker (1 or 2). Three steps are involved in determining whether linkage exists and, if so, estimating the distance between the gene and the known marker.

1. Establish linkage phase between the disease-producing allele of the gene and an allele of the marker in the family.
2. Determine if linkage exists between the 2 alleles.
3. If linkage exists, estimate the recombination frequency.

A family in which a mutation causing neurofibromatosis type 1 is transmitted in 3 generations. The genotype of a marker locus is shown for each pedigree member.

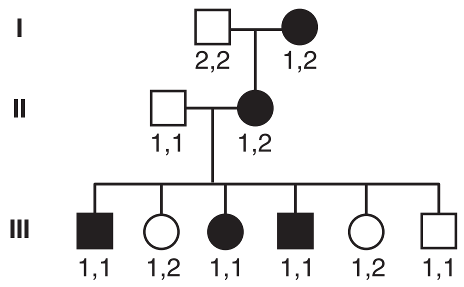
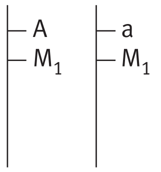


Figure II-5-4. Pedigree for Neurofibromatosis Type 1

Note

A **haplotype** is the combination of alleles on a single chromosome. With respect to the gene and the marker in Figure II-5-3, individual II-2 has two haplotypes, AM_1 and aM_2, depicted below, where A and a are alleles of the gene causing the disease. The marker has alleles 1 and 2.



Linkage Phase. The pedigree indicates that the grandmother (I-2) had the disease-producing allele (A) of the gene, which she passed to her daughter (II-2). Is it also possible to determine which allele of the marker was passed from the grandmother to her daughter? Yes, allele 1. If linkage is present, the disease-producing allele (A) is linked to allele 1 of the marker. We can then designate the daughter's 2 haplotypes as AM_1/aM_2, indicating the chromosomes from her mother/father respectively.

Determine If Linkage Exists. Are the gene and the marker actually linked as we hypothesize? Looking at the children in generation III (each representing a meiotic event in their mother, II-2), we would expect a child who inherited marker allele 1 from the mother to have the disease. The children who inherited allele 2 from the mother should not have the disease. Examination of the 6 children's haplotypes shows that this assumption is true in all but one case (III-6). Because

the AM_1 and aM_2 haplotypes remain together more than 50% of the time (or, conversely, are separated by recombination less than 50% of the time), our hypothesis of linkage is correct.

Estimate the Recombination Frequency. Out of 6 children, there is only one recombinant (III-6). The estimated recombination frequency in this family is 1/6, or 17%.

Recombination frequencies can be related to physical distance by the centimorgan (cM)

The recombination frequency provides a measure of genetic distance between any pair of linked loci. This distance is expressed in centimorgans. The centimorgan is equal to 1% recombination frequency. For example, if two loci show a recombination frequency of 2%, they are said to be 2 centimorgans apart. Physically, 1 cM is approximately equal to 1 million base pairs of DNA (1 Mb). This relationship is only approximate, however, because crossover frequencies are somewhat different throughout the genome, e.g., they are less common near centromeres and more common near telomeres.

Note

For Linked Loci:

- 1 centimorgan (cM) = 1% recombination frequency
- 1 cM ≈ 1 million base pairs

Determining Recombination Frequency Accurately: LOD Scores

In the previous example, a very small population (6 children, or 6 meiotic events) was used to determine and calculate linkage, allowing only a very rough estimate of linkage distance. In fact, there is some small chance that the gene and the marker are not actually linked at all and the data were obtained by chance. We could be more confident that our conclusions were correct if we had used a much larger population. Because families don't have 100 or 200 children, the next best approach is to combine data from different families with this same disease to increase the number of meioses examined. These data can be combined by using LOD (log of the odds) calculations.

A LOD score, calculated by computer, compares the probability (P) that the data resulted from actual linkage with a recombination frequency of theta (θ) versus the probability that the gene and the marker are unlinked (θ = 50%) and that the data were obtained by chance alone. In the example calculation:

$$\text{Odds} = \frac{\text{Probability of observing pedigree data if } \theta = 0.17}{\text{Probability of observing pedigree data if } \theta = 0.5}$$

In practice, because 17% might not be the correct number, the computer calculates these probabilities assuming a variety of recombination frequencies from θ = 0 (gene and marker are in the same location) to θ = 0.5 (gene and marker are unlinked). The "odds of linkage" is simply the probability that each recombination frequency (θ) is consistent with the family data. If data from multiple families are combined, the numbers can be added by using the $\log_{10}$ of these odds.

$$\text{Log of the Odds (LOD)} = \log_{10} \frac{\text{P (linkage at recombination frequency, } \theta)}{\text{P (unlinked, recom bination frequency, 50\%)}}$$

This equation need not be memorized. These calculations are done by computer and are displayed as a LOD table that gives the LOD score for each recombination frequency, θ.

Note

LOD score >3 indicates linkage, while score <−2 indicates no linkage.

To determine the recombination frequency:

- First examine the row of LOD scores.
- The highest LOD score >3 is the estimate of the recombination frequency.
- Then examine the row of recombination frequencies. The value directly above the LOD score >3 is the recombination frequency.

Table II-5-1. LOD Scores for a Gene and a Marker

Recombination frequency (θ)	0.01	0.05	0.10	0.20	0.30	0.40
LOD score	0.58	1.89	3.47	2.03	−0.44	−1.20

When interpreting LOD scores, the following rules apply:

- LOD score >3.00 shows statistical evidence of linkage. (It is 1,000 times more likely that the gene and the marker are linked at that distance than unlinked.)
- LOD score <–2.00 shows statistical evidence of nonlinkage. (It is 100 times more likely that the gene and the marker are unlinked than linked at that distance.)
- LOD score between –2.00 and 3.00 is indeterminate.

An examination of Table II-5-1 shows that in only one case is there convincing evidence for linkage and that score has a recombination frequency of 0.10. Therefore, the most likely distance between the gene and the marker is a recombination frequency of 10%, or 10 cM.

If no LOD score on the table is >3.00, the data may be suggestive of linkage, but results from additional families with the disease would need to be gathered.

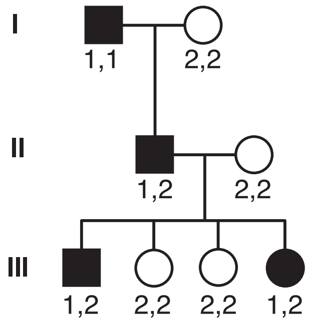
Gene mapping by linkage analysis serves several important functions:

- It can define the approximate location of a disease-causing gene.
- Linked markers can be used along with family pedigree information for genetic testing (*see* Chapter 6). In practice, markers that are useful for genetic testing must show less than 1% recombination with the gene involved (be <1 cM distant from the gene).
- Linkage analysis can identify locus heterogeneity.

Review Questions

Select the ONE best answer.

1. A family with an autosomal dominant disorder is typed for a 2 allele marker, which is closely linked to the disease locus. Based on the individuals in Generation III, what is the recombination rate between the disease locus and the marker locus?

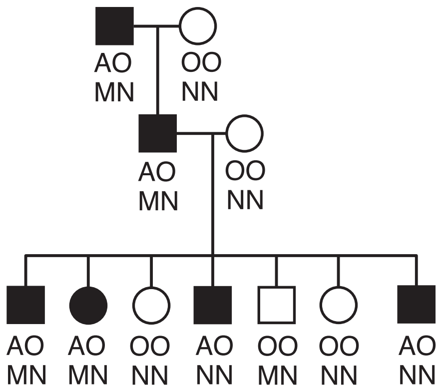


   A. 0
   B. 0.25
   C. 0.50
   D. 0.75
   E. 1.0
   F. The marker is uninformative

2. A man who has alkaptonuria marries a woman who has hereditary sucrose intolerance. Both are autosomal recessive diseases and both map to 3q with a distance of 10 cM separating the two loci. What is the chance they will have a child with alkaptonuria and sucrose intolerance?

   A. 0%
   B. 12.5%
   C. 25%
   D. 50%
   E. 100%

3. In a family study following an autosomal dominant trait through 3 generations, two loci are compared for their potential linkage to the disease locus. In the following 3-generation pedigree, shaded symbols indicate the presence of the disease phenotype, and the expression of ABO blood type and MN alleles are shown beneath each individual symbol.



Which of the following conclusions can be made about the linkage of the disease allele, ABO blood group locus, and MN locus?

A. The ABO and MN alleles are linked, but assort independently from the disease allele

B. The ABO, MN, and disease alleles all assort independently

C. The disease allele is linked to the ABO locus

D. The disease allele is linked to the ABO and MN loci

E. The disease allele is linked to the MN locus

Answers

1. **Answer: A.** In this pedigree, the disease allele is consistently transmitted with the 1 allele. There is no case in this small number of individuals where recombination between these two loci has occurred. Therefore, in Generation III, there is no recombination seen in any of the 4 individuals. Receiving the one allele always goes together with receiving the disease gene. Linked markers can be "uninformative" (**choice E**) in some pedigrees if, for example, the same alleles are expressed in all family members. In such a case, it would be impossible to determine any recombination frequency.

2. **Answer: A.** A child will inherit a gene for alkaptonuria from the father and the normal allele of this gene from the mother. Conversely, the child will inherit a gene for hereditary sucrose intolerance from the mother and a normal allele of this gene from the father. The child will therefore be a carrier for each disease but will not be affected with either one.

3. **Answer: C.** In this pedigree, the disease locus alleles are segregating with the ABO blood locus alleles. In each case, individuals who receive the A allele also receive the disease allele. The MN locus is not linked to the AO locus because individuals III-4, -5, and -7 are each recombinants between these loci. The MN locus is not linked to the disease allele because individuals III-4, -5, and -7 are each recombinants between these loci.

Genetic Diagnosis

6

Learning Objectives

- ❏ Understand concepts of direct and indirect genetic diagnosis
- ❏ Understand concepts of allele-specific oligonucelotides and dot blots

GENETIC DIAGNOSIS

Once a gene is identified, the associated genetic disease in at-risk individuals can be diagnosed.

The goal of genetic diagnosis is to determine whether an at-risk individual has inherited a disease-causing gene. Genetic diagnosis can be distinguished into 2 types:

- **Direct diagnosis**: the mutation itself is examined
- **Indirect diagnosis**: linked markers are used to infer whether the individual has inherited the chromosome segment containing the disease-causing mutation

Direct Diagnosis

High-Yield

PCR and allele-specific oligonucleotide (ASO) probes

ASO probes are short nucleotide sequences that bind specifically to a single allele of the gene. For example, the most common mutation causing hemochromatosis is the C282Y mutation that results from a G to A substitution in codon 282.

Codon:	280	281	282	283	284
Normal HFE allele:	TAT	ACG	T**G**C	CAG	GTG
			↑		
C282Y allele:	TAT	ACG	T**A**C	CAG	GTG
			↑		

The ASO for the normal allele would have the sequence

3′ ATA TGC A**C**G GTC CAC 5′

The ASO for the C282Y allele would have the sequence

3′ ATA TGC A**T**G GTC CAC 5′

The 2 ASOs could be used to probe the PCR-amplified material on a dot blot.

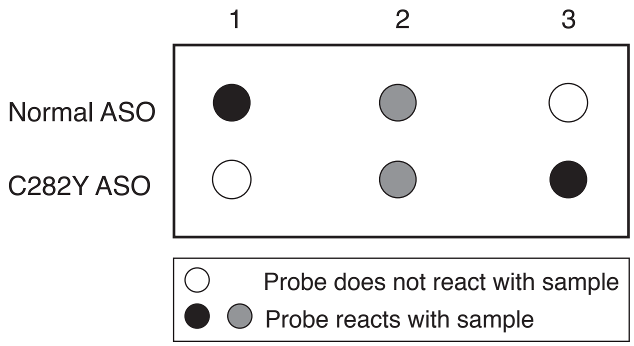


Figure II-6-1. Allele-Specific Oligonucleotide Probes in Hemochromatosis

The results show that individual 1 is homozygous for the normal HFE allele. Individual 2 is heterozygous for the normal and C282Y alleles. Individual 3 is homozygous for the C282Y allele. Only individual 3 would be expected to have symptoms. Note that this test merely determines genotype, and many considerations must be taken into account before predictions about phenotype could be made. Hemochromatosis has only about 15% penetrance, and in those who do have symptoms, variable expression is seen.

DNA chips

This approach involves embedding thousands of different oligonucleotides, representing various mutations and normal sequences, on a silicone chip. Patient DNA from specific regions is amplified by PCR, tagged with a fluorescent label, and exposed to the oligonucleotides on the chip. The sites of hybridization on the chip are recorded by a computer. This approach has the advantages of ready computerization and miniaturization (hundreds of thousands of oligonucleotides can be embedded on a single 2-cm^2 chip).

Restriction fragment length polymorphism (RFLP) analysis of PCR products (RFLP-PCR)

Occasionally a mutation that creates a disease-producing allele also destroys (or creates in some instances) a restriction enzyme site, as illustrated by the following case:

> A 14-year-old girl has been diagnosed with Gaucher disease (glucocerebrosidase A deficiency), an autosomal recessive disorder of sphingolipid catabolism. The mutation, T1448C, in this family also affects an HphI restriction site. PCR amplification of the area containing the mutation yields a 150-bp product. The PCR product from the normal allele of the gene is not cut by HphI. The PCR product of the mutant allele T1448C is cut by HphI to yield 114- and 36-bp fragments. The PCR product(s) is visualized directly by gel electrophoresis. Based on the results shown below in Figure II-6-3 using this assay on DNA samples from this family, what is the most likely conclusion about sibling 2?

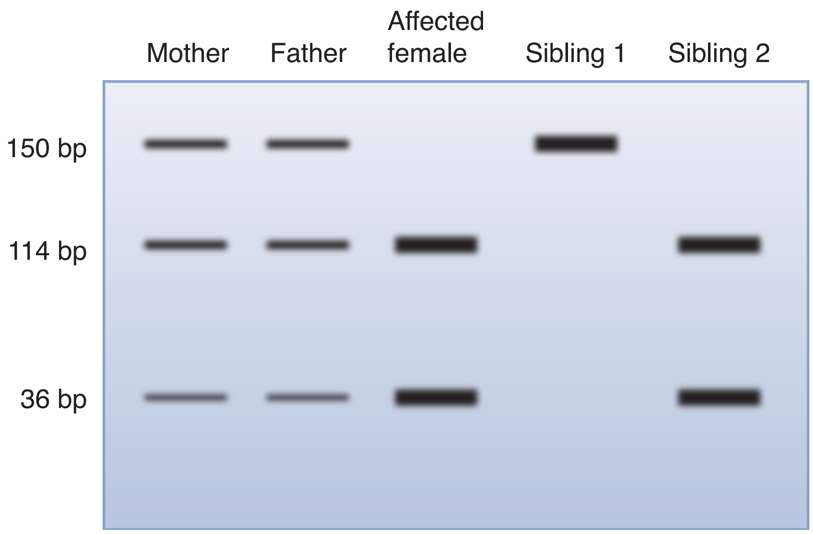


Figure II-6-2. PCR and RFLP for Gaucher Disease

(Ans.: Sibling 2 is also affected)

RFLP diagnosis of myotonic dystrophy

RFLP analysis is also useful in a few cases in which polymorphisms are too large to conveniently amplify with a PCR. One such case is myotonic dystrophy, in which the expanded sequence is within the gene region itself (a CTG in the 3′ untranslated region). This disease shows anticipation, and family members with a severe form of myotonic dystrophy may have several thousand copies of this repeat. As shown in Figure II-6-3, when *Eco*RI digests are analyzed by Southern blotting, a probe reveals 9- to 10-kb fragments in unaffected individuals. The size of the fragment can reach 20 kb in severely affected individuals.

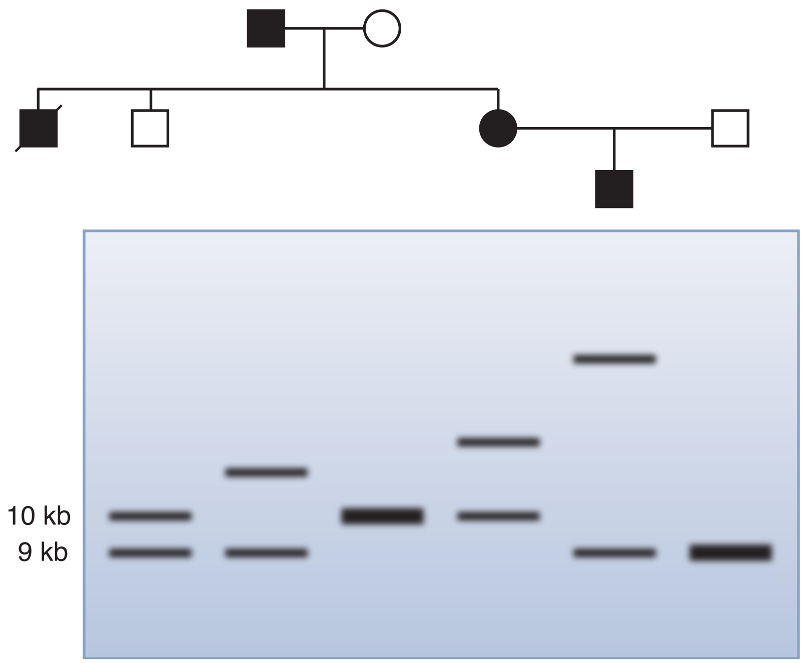


Figure II-6-3. *Eco*RI RFLP Analysis of a Family with Myotonic Dystrophy

Direct DNA sequencing

Sequencing of the entire gene (or at least the exons and the intron–exon boundaries) is time-consuming and expensive. However, it is sometimes necessary if no specific set of mutations is responsible for most cases of a disease (e.g., familial breast cancer caused by any of several hundred mutations of the *BRCA1* or *BRCA2* genes). DNA sequencing is typically done using automated sequencing machines.

Indirect Diagnosis

High-Yield

If the mutation causing a disease in a family is not known, indirect genetic analysis can be used to infer whether a parent has transmitted the mutation to his or her offspring. Indirect genetic analysis uses genetic markers that are closely linked (showing <1% recombination) to the disease locus. The markers are the same ones used in genetic mapping studies: restriction fragment length polymorphisms (RFLPs), short tandem repeat polymorphisms (STRPs), and single nucleotide polymorphisms (SNPs). Because STRPs can have multiple alleles (with each allele representing a different number of repeats), they are often informative markers to use.

Indirect genetic diagnosis using STRPs

Suppose there is a 3-generation family in which Marfan syndrome is being transmitted. Each family member has been typed for a 4-allele STRP that is closely linked to the disease locus. The affected father in generation I transmitted the disease-causing mutation to his daughter, and he also transmitted allele 3 of the marker. This allows us to establish linkage phase in this family.

Because of the close linkage between the marker and the disease locus, we can predict accurately that the offspring in generation III who receive allele 3 from their mother will also receive the disease-causing mutation. Thus, the risk for each child, instead of being the standard 50% recurrence risk for an autosomal dominant disease, is much more definitive: nearly 100% or nearly 0%.

The genotype of a closely linked marker locus is shown below each individual.

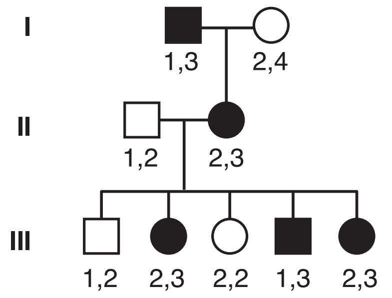


Figure II-6-4. Three-Generation Family in Which Marfan Syndrome Is Being Transmitted

Recurrence risks may have to take into account the small chance of recombination between the marker allele and the disease-causing gene. If the STR and the disease-causing gene used in this case show 1% recombination, then the recurrence risk for a fetus in generation III whose marker genotype is 2,2 would be 1% rather than 0%. If a fetus in generation III had the marker genotype 2,3, the recurrence risk for that child would be 99%.

Indirect genetic testing using RFLPs

If an RFLP is used as a marker for a disease-causing gene, the data may be analyzed by using Southern blotting and a probe for the gene region.

> A man and a woman seek genetic counseling because the woman is 8 weeks pregnant, and they had a previous child who died in the perinatal period. A retrospective diagnosis of long-chain acyl-CoA dehydrogenase (LCAD) deficiency was made based on the results of mass spectrometry performed on a blood sample. The couple also has an unaffected 4-year-old daughter with a normal level of LCAD activity consistent with homozygosity for the normal LCAD allele. The parents wish to know whether the current pregnancy will result in a child with the same rare condition as the previous child who died. DNA samples from both parents and their unaffected 4-year-old daughter are tested for mutations in the LCAD gene. All test negative for the common mutations. The family is then tested for polymorphism at a BamII site within exon 3 of the LCAD gene by using a probe for the relevant region of this exon. The RFLP marker proves informative. Fetal DNA obtained by amniocentesis is also tested in the same way. The results of the Southern blot are shown below. What is the best conclusion about the fetus?

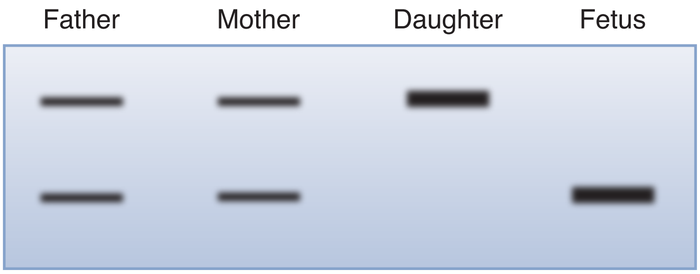


Figure II-6-5. RFLP Analysis for LCAD

(Answer: Fetus is homozygous for LCAD mutation and should be clinically affected)

Although RFLP analysis can be used as both an indirect test and a direct test, there is a significant difference between the two situations.

In the direct test, the mutation causing the disease is the same as the one that alters the restriction site. There is no distance separating the mutations and no chance for recombination to occur, which might lead to an incorrect conclusion.

In the indirect assay, the mutation in the restriction site (a marker) has occurred independently of the mutation causing the disease. Because the mutations are close together on the chromosome, the RFLP can be used as a surrogate marker for the disease-producing mutation. Linkage phase in each family must be established. Because the RFLP and the locus of the disease-producing mutation are some distance apart, there is a small chance for recombination and incorrect conclusions.

RFLP analysis for an X-linked disease

Individual II-2 in the family shown below has Lesch Nyhan disease. His sister, II-4, is pregnant and wants to know the likelihood that her child will be affected. The mutation in this family is uncharacterized, but is mapped to within 0.05 cM of an EcoR1 site that is informative in this family. DNA from all family members is obtained. Fetal DNA is obtained by chorionic villus sampling. What is the best conclusion about the fetus?

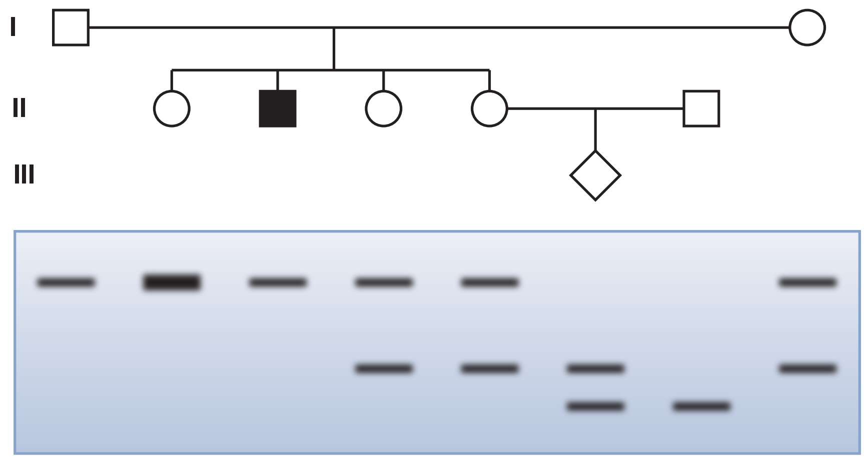


Figure II-6-6. RFLP Analysis of HGPRT Deficiency in a Family

(Answer: Fetus (a girl) will not be affected; nor will she be a carrier because her mother, II-4, is not a carrier)

Direct versus Indirect Genetic Diagnosis

Direct genetic diagnosis is used whenever possible. Its major limitation is that the disease-producing mutation(s) must be known if one is to test for them.

If a family carries a mutation not currently documented, as in the family above with LCAD deficiency, it will not be detected by direct mutation testing. In these cases, indirect genetic testing can be used.

Table II-6-1. Features of Indirect and Direct Genetic Diagnosis

	Indirect Diagnosis	Direct Diagnosis
Family information needed	Yes	No
Errors possible because of recombination	Yes	No
Markers may be uninformative	Yes	No
Multiple mutations can be assayed with a single test	Yes	No
Disease-causing mutation itself must be known	No	Yes

APPLICATIONS OF GENETIC DIAGNOSIS

Genetic diagnosis is used in a variety of settings, including the ones listed below.

- Carrier diagnosis in recessive diseases
- Presymptomatic diagnosis for late-onset diseases
- Asymptomatic diagnosis for diseases with reduced penetrance
- Prenatal diagnosis
- Preimplantation testing

Prenatal Genetic Diagnosis

Prenatal diagnosis is one of the most common applications of genetic diagnosis. Diagnosis of a genetic disease in a fetus may assist parents in making an informed decision regarding pregnancy termination and in preparing them emotionally and medically for the birth of an affected child. There are various types of prenatal diagnosis.

Amniocentesis

With amniocentesis, a small sample of amniotic fluid (10–20 mL) is collected at approximately 16 weeks' gestation. Fetal cells are present in the amniotic fluid and can be used to diagnose single-gene disorders, chromosome abnormalities, and some biochemical disorders. Elevated α-fetoprotein levels indicate a fetus with a neural tube defect. The risk of fetal demise due to amniocentesis is estimated to be approximately 1/200.

Chorionic villus sampling

This technique, typically performed at 10–12 weeks' gestation, involves the removal of a small sample of chorionic villus material (either a transcervical or a transabdominal approach may be used). The villi are of fetal origin and thus provide a large sample of actively dividing fetal cells for diagnosis. This technique has the advantage of providing a diagnosis earlier in the pregnancy. There is a small possibility of diagnostic error because of placental mosaicism (i.e., multiple cell types in the villi). The risk of fetal demise is higher than with amniocentesis (about 1/100).

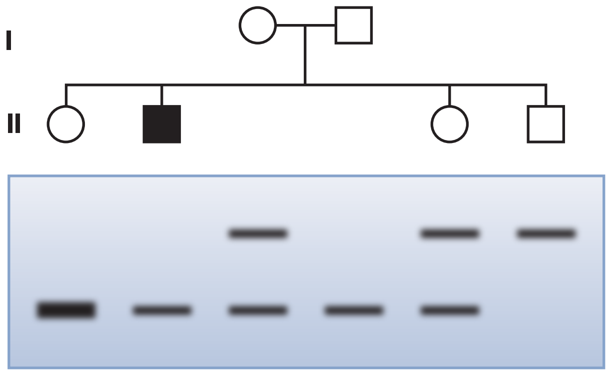
Preimplantation diagnosis

Embryos derived from *in vitro* fertilization can be diagnosed by removing a single cell, typically from the eight-cell stage (this does not harm the embryo). DNA is PCR amplified and is used to make a genetic diagnosis. The advantage of this technique is that pregnancy termination need not be considered: only embryos without the mutation are implanted. There is a possibility of diagnostic error as a result of PCR amplification from a single cell.

Review Questions

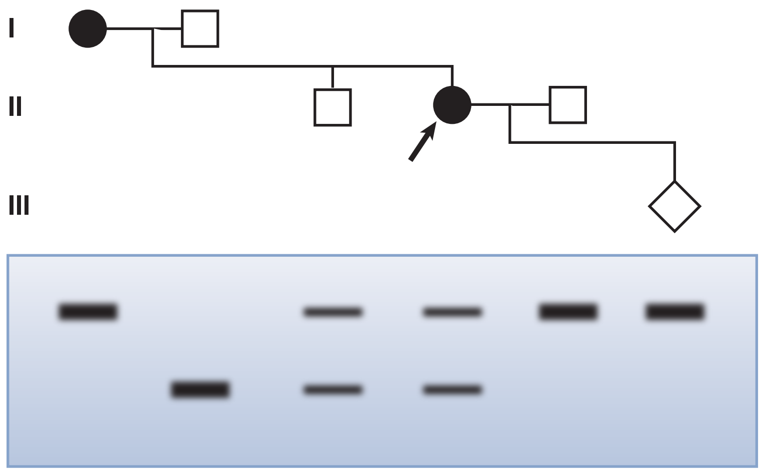
Select the ONE best answer.

1. The pedigree below shows a family in which hemophilia A, an X-linked disorder, is segregating. PCR products for each member of the family are also shown for a short tandem repeat polymorphism located within an intron of the factor VIII gene. What is the best explanation for the phenotype of individual II-1?



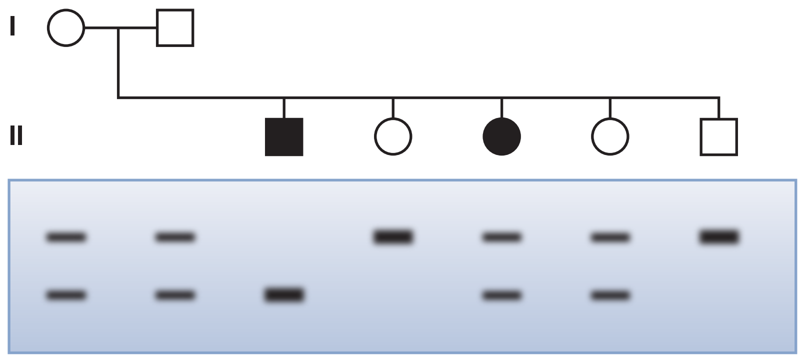
A. Heterozygous for the disease-producing allele
B. Homozygous for the disease-producing allele
C. Homozygous for the normal allele
D. Incomplete penetrance
E. Manifesting heterozygote

2. A 22-year-old woman with Marfan syndrome, a dominant genetic disorder, is referred to a prenatal genetics clinic during her tenth week of pregnancy. Her family pedigree is shown below (the arrow indicates the pregnant woman). PCR amplification of a short tandem repeat (STR) located in an intron of the fibrillin gene is carried out on DNA from each family member. What is the best conclusion about the fetus (III-1)?



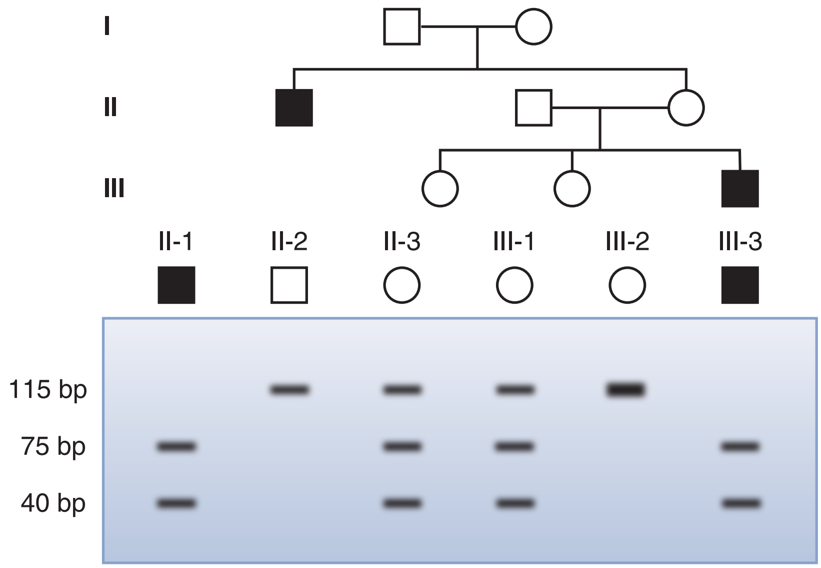
A. Has a 25% change of having Marfan syndrome
B. Has a 50% chance of having Marfan syndrome
C. Will develop Marfan syndrome
D. Will not develop Marfan syndrome
E. Will not develop Marfan syndrome, but will be a carrier of the disease allele

3. The pedigree below represents a family in which phenylketonuria (PKU), an autosomal recessive disease, is segregating. Southern blots for each family member are also shown for an RFLP that maps 10 million bp upstream from the phenylalanine hydroxylase gene. What is the most likely explanation for the phenotype of II-3?



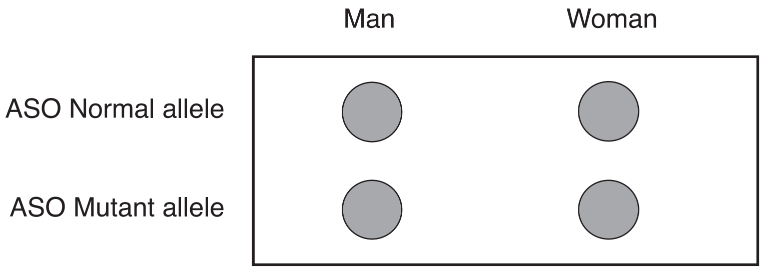
A. A large percentage of her cells have the paternal X chromosome carrying the PKU allele active
B. Heteroplasmy
C. Male I-2 is not the biologic father
D. PKU shows incomplete penetrance
E. Recombination has occurred

4. A 14-year-old boy has Becker muscular dystrophy (BMD), an X-linked recessive disease. A maternal uncle is also affected. His sisters, aged 20 and 18, wish to know their genetic status with respect to the BMD. Neither the boy nor his affected uncle has any of the known mutations in the dystrophin gene associated with BMD. Family members are typed for a *Hin*dII restriction site polymorphism that maps to the 5′ end of intron 12 of the dystrophin gene. The region around the restriction site is amplified with a PCR. The amplified product is treated with the restriction enzyme *Hin*dII and the fragments separated by agarose gel electrophoresis. The results are shown below. What is the most likely status of individual III-2?



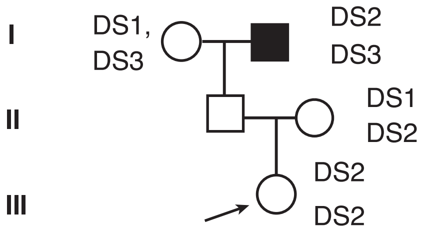
A. Carrier of the disease-producing allele
B. Hemizygous for the disease-producing allele
C. Homozygous for the normal allele
D. Homozygous for the disease-producing allele
E. Manifesting heterozygote

5. Two phenotypically normal second cousins marry and would like to have a child. They are aware that one ancestor (great-grandfather) had PKU and are concerned about having an affected offspring. They request ASO testing and get the following results. What is the probability that their child will be affected?



A. 1.0
B. 0.75
C. 0.67
D. 0.50
E. 0.25

6. A 66-year-old man (I-2) has recently been diagnosed with Huntington disease, a late-onset, autosomal dominant condition. His granddaughter (III-1) wishes to know whether she has inherited the disease-producing allele, but her 48-year-old father (II-1) does not wish to be tested or to have his status known. The grandfather, his unaffected wife, the granddaughter, and her mother (II-2) are tested for alleles of a marker closely linked to the *huntingtin* gene on 4p16.3. The pedigree and the results of testing are shown below. What is the best information that can be given to the granddaughter (III-1) about her risk for developing Huntington disease?



A. 50%
B. 25%
C. Marker is not informative
D. Nearly 100%
E. Nearly 0%

Answers

1. **Answer: A.** The female II-1 in this family is heterozygous for the marker (from the gel) and also has an unaffected father. Her mother is a carrier and the bottom band in the mother's pattern is associated with the disease-producing allele of the factor VIII gene. All observations are consistent with II-1 being heterozygous (Xx) for the factor VIII gene. She has no symptoms, so she is not a manifesting heterozygote (**choice E**). She cannot be homozygous for the disease-producing allele (**choice B**) because her father is unaffected. Homozygosity for the normal allele (**choice C**) is inconsistent with the results shown on the gel. She has inherited the chromosome from her mother (bottom band) that carries the mutant factor VIII allele, but from her father she has received a chromosome carrying the normal allele. Note that her father is not affected, and the bottom band in his pattern is in linkage phase with the normal allele of the gene. This is a case where linkage phase is different in the mother and the father. Incomplete penetrance (**choice D**) is not a good choice because the female (II-1) does not have the disease-producing genotype. She is heterozygous for the recessive and (dominant) normal allele. One would expect from her genotype that she would be unaffected.

2. **Answer: C.** The blot shows the top band in the patterns of I-1 and II-2 (the proband) is associated with the disease-producing allele. Because the fetus has inherited this marker allele from the mother (II-2) and Marfan disease is dominant, the fetus will develop Marfan disease. **Choices A** and **B** are recurrence risks associated with the pedigree data. With no blot to examine, choice B, 50% risk would be correct. **Choice D** would be correct if the blot from the fetal DNA showed both the bottom band (must be from mother) and the top band (from the unaffected father). **Choice E** is incorrect because Marfan is a dominant disease with no "carrier" status.

3. **Answer: E.** Although II-3 has an RFLP pattern consistent with heterozygosity for the PKU allele, she has PKU. The best explanation offered is that recombination has occurred, and although she is heterozygous for the restriction site generating the RFLP pattern, she is homozygous for the mutation causing PKU. The restriction site is 10 million bp upstream from the phenylalanine hydroxylase gene so there is a minimum chance of recombination of 10%. Although this is small, it is the most likely of the options listed. The phenylalanine hydroxylase gene is not on the X chromosome (**choice A**). Heteroplasmy (**choice B**) is associated with mitochondrial pedigrees, and the phenylalanine hydroxylase gene is a nuclear one. The RFLP pattern is quite consistent with I-2 being the biologic father (**choice C**), and he is a known carrier of the PKU mutation because he has another affected child (II-1). If II-3's RFLP pattern showed homozygosity for the marker (identical to II-1), and she had no symptoms, incomplete penetrance (**choice D**) would be a good choice.

4. **Answer: C.** The disease-producing allele of the gene is associated with the presence of the *Hin*dII site. Notice that both affected males show two smaller bands (75 and 40 bp). II-3, a carrier female, also has these two smaller bands in her pattern, in addition to a larger PCR product (115 bp), representing the absence of the *Hin*dII site on her normal chromosome. III-2 has only the larger PCR product (notice the density because both chromosomes yielded this product). She is homozygous for the normal allele. **Choice A**, carrier, would be correct if her pattern had looked like those of II-3 and III-1. All the males shown are hemizygous (**choice B**) for the dystrophin gene because they have only one copy. II-1 and III-3 are hemizygous for the disease-producing allele, and II-2 is hemizygous for the normal allele. No one in the family is homozygous for the disease-producing allele (**choice D**). In an X-linked pattern, this would be characteristic of a female with two copies of the disease-producing allele and is very rarely seen. III-2 is not a manifesting heterozygote (**choice E**) because she has no symptoms and is not a heterozygote.

5. **Answer: E.** The blot indicates that both parents are heterozygous for the mutant allele. Because both are phenotypically normal, the disease must be autosomal recessive. If it had been X-linked recessive, the man would be hemizygous. Thus, the chance they will have an affected child is 25% (0.25).

6. **Answer: A.** The affected grandfather has marker alleles DS2 and DS3. There is no information about which one is in linkage phase with his diseaseproducing *huntingtin* allele. On the basis of the pedigree alone, the daughter has a 25% change of inheriting the grandfather's disease-producing *huntingtin* allele **(choice B)**; however, she would like more information. Because her father (II-1) does not wish to be tested or know about his genetic status with respect to Huntington's, it is unethical to test the daughter for the triplet repeat expansion. The results would necessarily reveal the status of her father also. By doing an indirect genetic test, one can see the daughter has inherited one of her marker alleles (DS2) from the grandfather via her father. This means she has a 50% chance of developing Huntington's because there is a 50% chance that DS2 is a marker for the disease-producing *huntingtin* allele in the grandfather and a 50% chance it is not (and DS3 is). Notice the result does not reveal additional information about her father (II-1). Before her testing, he had a 50% chance of having the disease-producing *huntingtin* allele. His risk is still 50% with the information from the daughter's test. However, if the father (II-1) does develop Huntington's in the future, that will mean that the daughter has a 100% chance of having the disease also **(choice D)**.

   If her marker status had been DS1/DS1, her chances of developing Huntington's would have been near 0 (**choice E**) because she did not inherit these alleles from her grandfather. One came from her grandmother (via her father) and one from her mother. This result still would not reveal additional relevant information about her father (II-1), whose risk would remain 50%.

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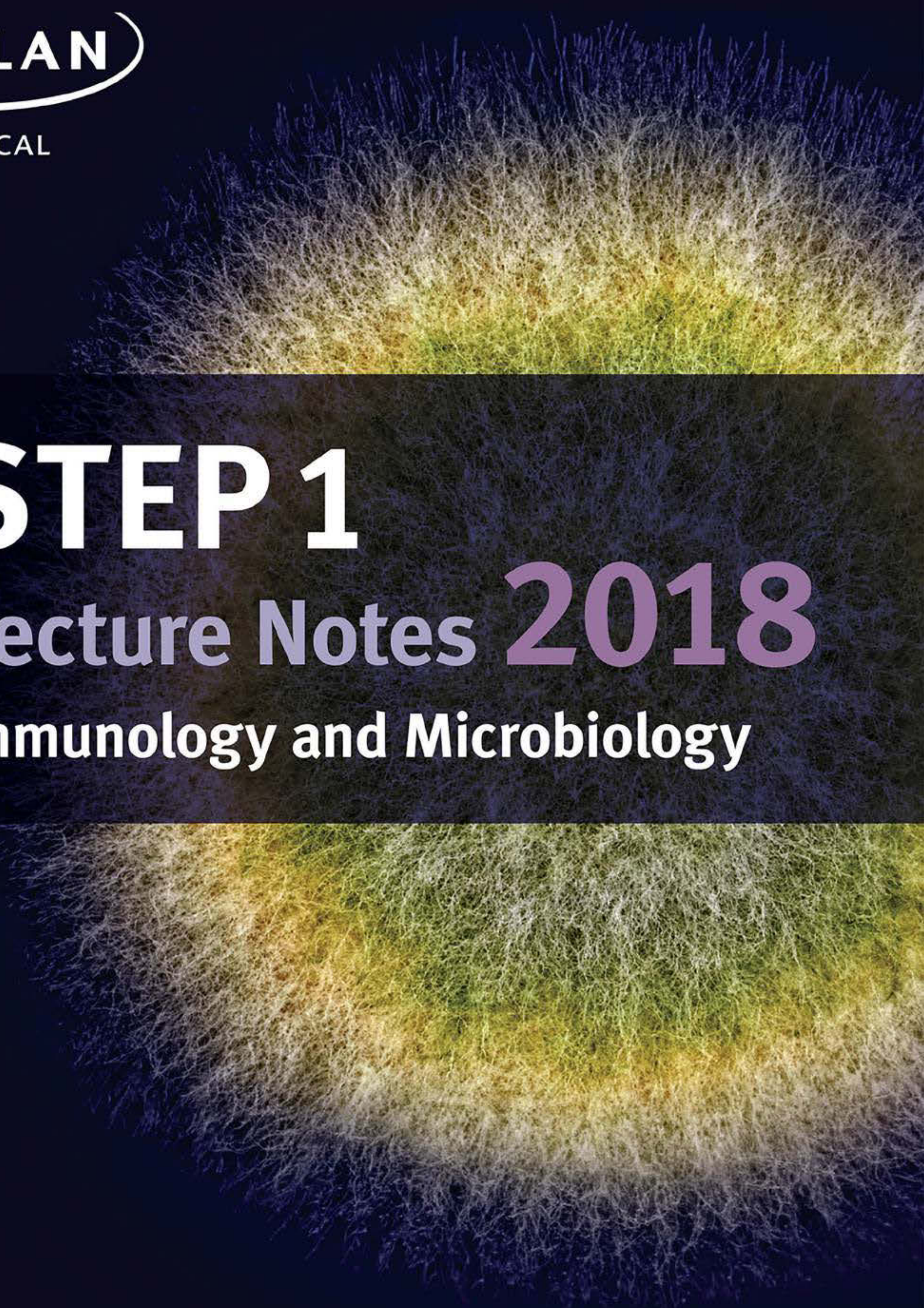

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PART I

Immunology

The Immune System 1

Learning Objectives

- ❑ Define and describe the components of the immune system
- ❑ Discriminate between innate and acquired immunity

THE IMMUNE SYSTEM

The immune system is designed to recognize and respond to non-self antigen in a coordinated manner. Additionally, cells that are diseased, damaged, distressed or dying are recognized and eliminated by the immune system.

The immune system is divided into 2 complementary arms: the **innate** and the **adaptive** immune systems.

Innate Immunity

Innate immunity provides the body's first line of defense against infectious agents. It involves several defensive barriers:

- Anatomic and physical (skin, mucous membranes and normal flora)
- Physiologic (temperature, pH, anti-microbials and cytokines)
- Complement
- Cellular: phagocytes and granulocytes
- Inflammation

Innate immune defenses have the following characteristics in common:

- Are **present intrinsically** with or without previous stimulation
- Have **limited specificity** for shared microbe and cellular structures (pathogen-associated molecular patterns [PAMPs] and damage-associated molecular patterns [DAMPs])
- Have **limited diversity** as reflected by a limited number of pattern recognition receptors
- Are not enhanced in activity upon subsequent exposure—**no memory**

Adaptive Immunity

The components of the adaptive immune response are B and T lymphocytes and their effector cells.

Adaptive immune defenses have the following characteristics in common:

- Each B and T lymphocyte is **specific** for a particular antigen
- As a population, lymphocytes have extensive diversity
- Are enhanced with each repeat exposure—**immunologic memory**
- Are capable of **distinguishing self** from **non-self**
- Are **self-limiting**

The features of adaptive immunity are designed to give the individual the best possible defense against disease.

- **Specificity** is required, along with **immunologic memory,** to protect against persistent or recurrent challenge.
- **Diversity** is required to protect against the maximum number of potential pathogens.
- **Specialization** of effector function is necessary so that the most effective defense can be mounted against diverse challenges.
- The ability to **distinguish between self** (host cells) **and non-self** (pathogens) is vital in inhibiting an autoimmune response.
- **Self-limitation** allows the system to return to a basal resting state after a challenge to conserve energy and resources and to avoid uncontrolled cell proliferation resulting in leukemia or lymphoma.

Table I-1-1. Innate versus Adaptive Immunity

Characteristics	Innate	Adaptive
Specificity	For pathogen-associated molecular patterns (PAMPs)	For specific antigens of microbial and nonmicrobial agents
Diversity	Limited	High
Memory	No	Yes
Self-reactivity	No	No
Components		
Anatomic and physiologic barriers	Skin, mucosa, normal flora, temperature, pH, antimicrobials, and cytokines	Lymph nodes, spleen, mucosal-associated lymphoid tissues
Blood proteins	Complement	Antibodies
Cells	Phagocytes, granulocytes and natural killer (NK) cells	B lymphocytes and T lymphocytes

Function

The innate and adaptive arms of the immune response work in collaboration to stop an infection. Once a pathogen has broken through the anatomic and physiologic barriers, the innate immune response is immediately activated, oftentimes it is able to contain and eliminate the infection.

When the innate immune response is unable to control the replication of a pathogen, the adaptive immune response is engaged and activated by the innate immune response in an antigen-specific manner. Typically, it takes 1-2 weeks after the primary infection for the adaptive immune response to begin clearance of the infection through the action of effector cells and antibodies.

Once an infection has been cleared, both the innate and adaptive immune responses cease. Antibodies and residual effector cells continue to provide protective immunity, while memory cells provide long-term immunologic protection from subsequent infection.

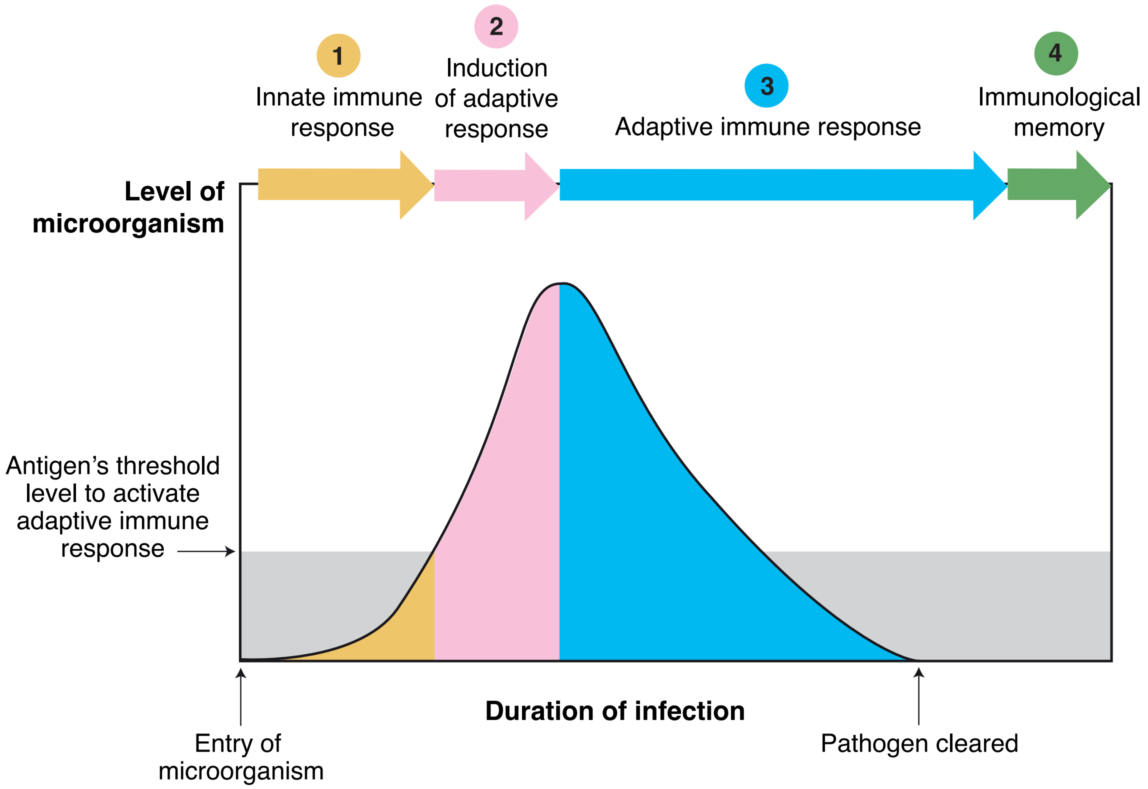


Figure I-1-1. Timeline of the Immune Response to an Acute Infection

The innate and adaptive immune responses do not act independently of one another; rather, they work by a positive feedback mechanism.

- Phagocytic cells recognize pathogens by binding PAMPs through various pattern-recognition receptors leading to phagocytosis.
- Phagocytic cells process and present antigen to facilitate stimulation of specific T lymphocytes with subsequent release of cytokines that trigger initiation of specific immune responses.
- T lymphocytes produce cytokines that enhance microbicidal activities of phagocytes.
- Cytokines released by phagocytes and T lymphocytes will drive differentiation of B lymphocytes into plasma cells and isotype switching.
- Antibodies will aid in the destruction of pathogen through opsonization, complement activation and antibody-dependent cellular cytotoxicity.

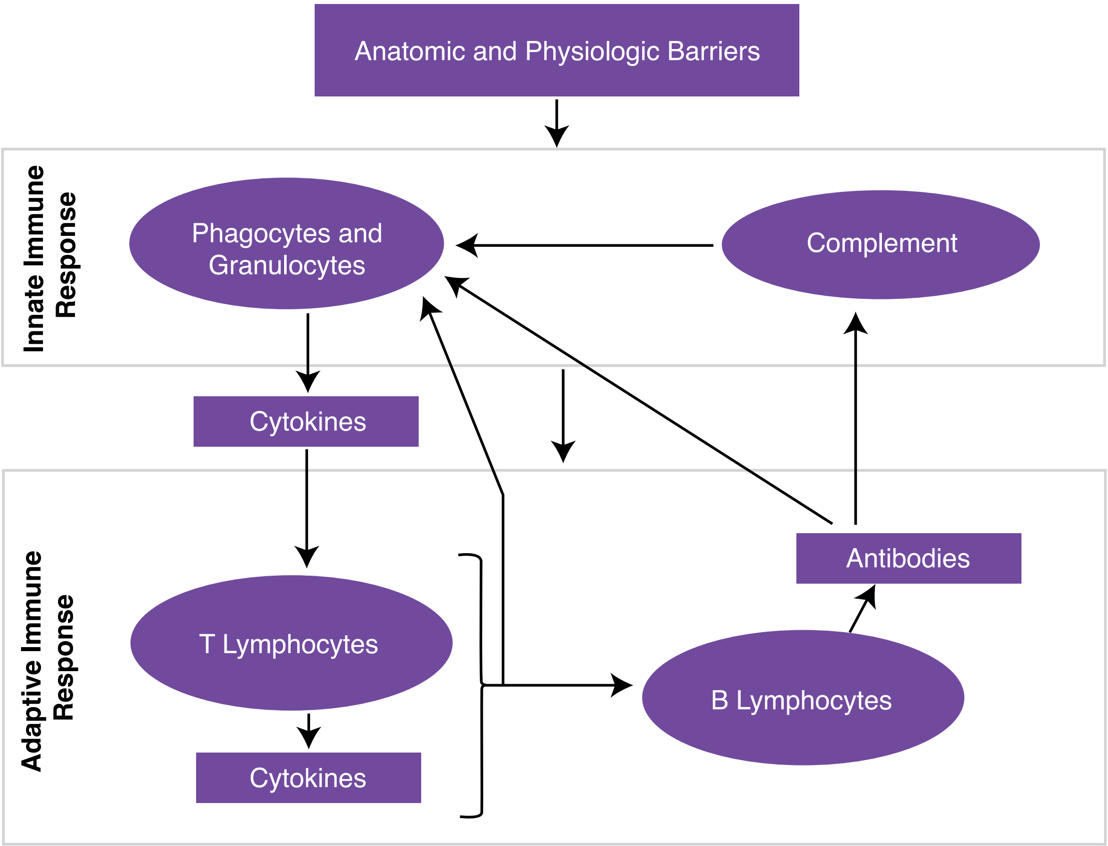


Figure I-1-2. Interaction between Innate and Adaptive Immune Responses

Ontogeny of the Immune Cells 2

Learning Objectives

- ❏ Explain information related to origin and function of cells of the immune system
- ❏ Explain information related to antigen recognition molecules of lymphocytes
- ❏ Answer questions about the generation of receptor diversity

ORIGIN

Hematopoiesis involves the production, development, differentiation, and maturation of the blood cells (erythrocytes, megakaryocytes and leukocytes) from **multipotent stem cells**. The site of hematopoiesis changes during development.

During embryogenesis and early fetal development, the yolk sac is the site of hematopoiesis. Once organogenesis begins, hematopoiesis shifts to the liver and spleen, and finally, to the bone marrow where it will remain throughout adulthood.

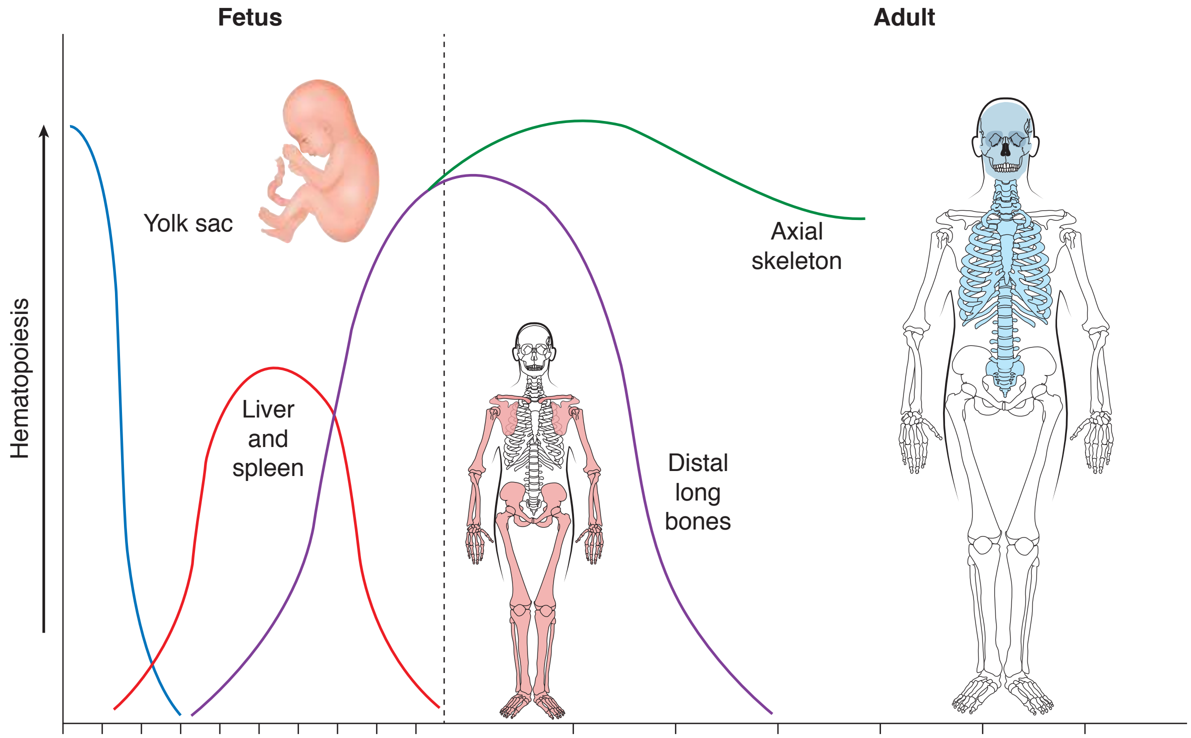


Figure I-2-1. Sites of Hematopoiesis during Development

These **multipotent stem cells** found in the bone marrow have the ability to undergo asymmetric division. One of the 2 daughter cells will serve to renew the population of stem cells (**self-renewal**), while the other can give rise to either a common lymphoid progenitor cell or a common myeloid progenitor cell (**potency**). The multipotent stem cells will differentiate into the various lymphoid and myeloid cells in response to various cytokines and growth factors.

- The **common lymphoid progenitor cell** gives rise to B lymphocytes, T lymphocytes and natural killer (NK) cells.
- The **common myeloid progenitor cell** gives rise to erythrocytes, megakaryocytes/thrombocytes, mast cells, eosinophils, basophils, neutrophils, monocytes/macrophages and dendritic cells.

FUNCTION

The white blood cells of both the myeloid and lymphoid stem cells have specialized functions in the body once their differentiation in the bone marrow is complete. Cells of the myeloid lineage, except erythrocytes and megakaryocytes, perform non-specific, stereotypic responses and are members of the innate branch of the immune response. B lymphocytes and T lymphocytes of the lymphoid lineage perform focused, antigen-specific roles in immunity. Natural killer cells are also from the lymphoid lineage but participate in innate immunity.

Although B lymphocytes and T lymphocytes in the bloodstream are almost morphologically indistinguishable at the light microscopic level, they represent 2 interdependent cell lineages.

- **B lymphocytes** remain within the **bone marrow** to complete their development.
- **T lymphocytes** leave the bone marrow and undergo development within the **thymus**.

Both B and T lymphocytes have surface membrane receptors designed to bind to specific antigens; the generation of these receptors will be discussed in chapter 4.

- The **natural killer (NK) cell** (the third type of lymphocyte) is a large granular lymphocyte that recognizes tumor and virally infected cells through non-specific binding.

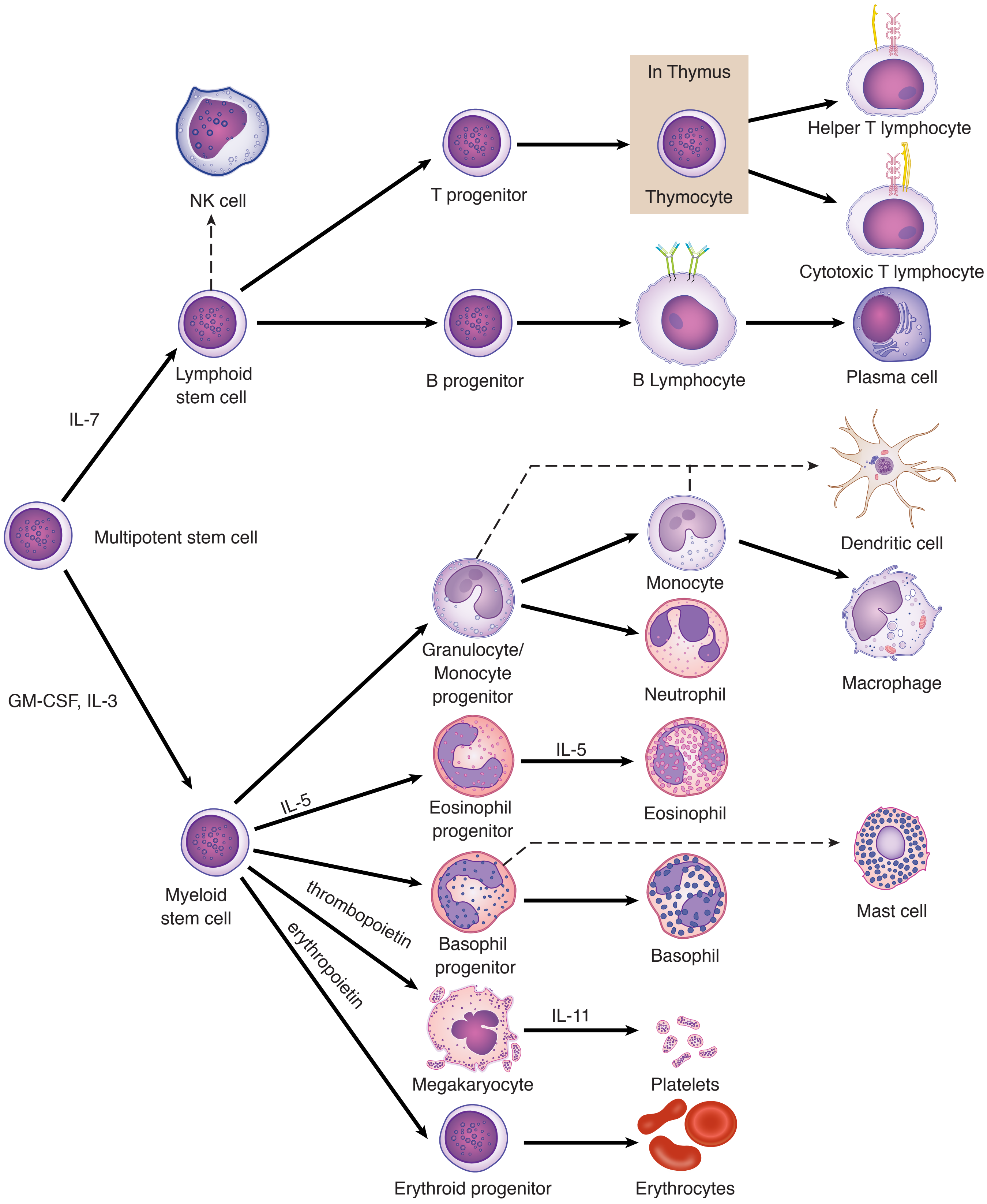
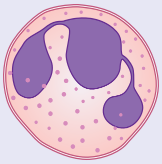
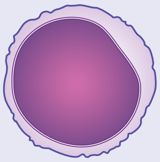
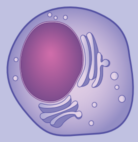
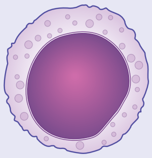


Figure I-2-2. Ontogeny of Immune Cells

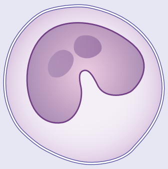
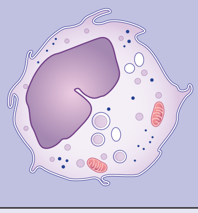
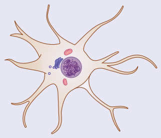
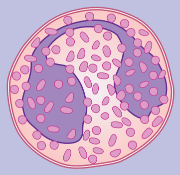
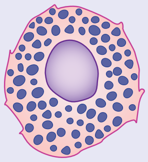
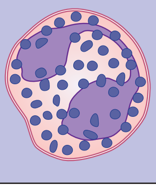
Table I-2-1. White Blood Cells

(Order is based on relative percentages as they appear in the blood)

Myeloid Cell	Tissue Location	Physical Description	Function
Neutrophil or polymorpho-nuclear (PMN) cell 	Most abundant circulating blood cell	Granulocyte with a segmented, lobular nuclei (3–5 lobes) and small pink cyto-plasmic granules	Phagocytic activity aimed at killing extracellular pathogens
Lymphoid Cell	**Tissue Location**	**Physical Description**	**Function**
Lymphocyte 	Bloodstream, secondary lym-phoid tissues	Large, dark-staining nucleus with a thin rim of cytoplasm Surface markers: B lymphocytes – CD19, 20, 21 T lymphocytes – CD3 Helper T cells – CD4 CTLs – CD8	No function until activated in the secondary lymphoid tissues
Plasma cell 	Bloodstream, sec-ondary lymphoid tissue and bone marrow	Small eccentric nucleus, intensely staining Golgi ap-paratus	Terminally differentiated B lymphocyte that secretes antibodies
Natural killer cell 	Bloodstream	Lymphocyte with large cytoplasmic granules Surface markers: CD16, 56	Kills virally infected cells and tumor cells

(Continued)

Table I-2-1. White Blood Cells (*cont'd*)

Myeloid Cell	Tissue Location	Physical Description	Function
Monocyte 	Circulating blood cell	Agranulocyte with a bean or kidney-shaped nucleus	Precursor of tissue macrophage
Macrophage 	Resident in all tissues	Agranulocyte with a ruffled cytoplasmic membrane and cytoplasmic vacuoles and vesicles	• Phagocyte • Professional antigen presenting cell • T-cell activator
Dendritic cell 	Resident in epithelial and lymphoid tissue	Agranulocyte with thin, stellate cytoplasmic projections	• Phagocyte • Professional antigen presenting cell • T-cell activator
Eosinophil 	Circulating blood cell recruited into loose connective tissue of the respiratory and GI tracts	Granulocyte with bilobed nucleus and large pink cytoplasmic granules	• Elimination of large extracellular parasites • Type I hypersensitivity
Mast cell 	Reside in most tissues adjacent to blood vessels	Granulocyte with small nucleus and large blue cytoplasmic granule	• Elimination of large extracellular parasites • Type I hypersensitivity
Basophil 	Low frequency circulating blood cell	Granulocyte with bilobed nucleus and large blue cytoplasmic granules	• Elimination of large extracellular parasites • Type I hypersensitivity

Laboratory evaluation of patients commonly involves assessment of white blood cell morphology and relative counts by examination of a blood sample. Changes in the morphology and proportions of white blood cells indicate the presence of some pathologic state. A standard white blood cell differential includes neutrophils, band cells, lymphocytes (B lymphocytes, T lymphocytes, and NK cells), monocytes, eosinophils and basophils.

Table I-2-2. Leukocytes Evaluated in a WBC Differential

Cell Type	Adult Reference Range (%)
Neutrophils (PMNs)	50–70
Band cells	0–5
Lymphocytes	20–40
Monocytes	5–10
Eosinophils	0–5
Basophils	<1

Lymphocyte Development and Selection

3

Learning Objectives

- ❑ Answer questions about selection of T and B lymphocytes
- ❑ Solve problems concerning innate immunity and components/barriers

ANTIGEN RECOGNITION MOLECULES OF LYMPHOCYTES

Each cell of the lymphoid lineage is clinically identified by the characteristic surface molecules that it possesses.

- The mature, naïve **B lymphocyte**, in its mature ready-to-respond form, expresses 2 isotypes of antibody or immunoglobulin called IgM and IgD within its surface membrane.
- The mature, naive **T cell** expresses a single genetically related molecule, called the **T-cell receptor** (**TCR**), on its surface.

Both of these types of antigen receptors are encoded within the immunoglobulin superfamily of genes and are expressed in literally millions of variations in different lymphocytes as a result of complex and random rearrangements of the cells' DNA.

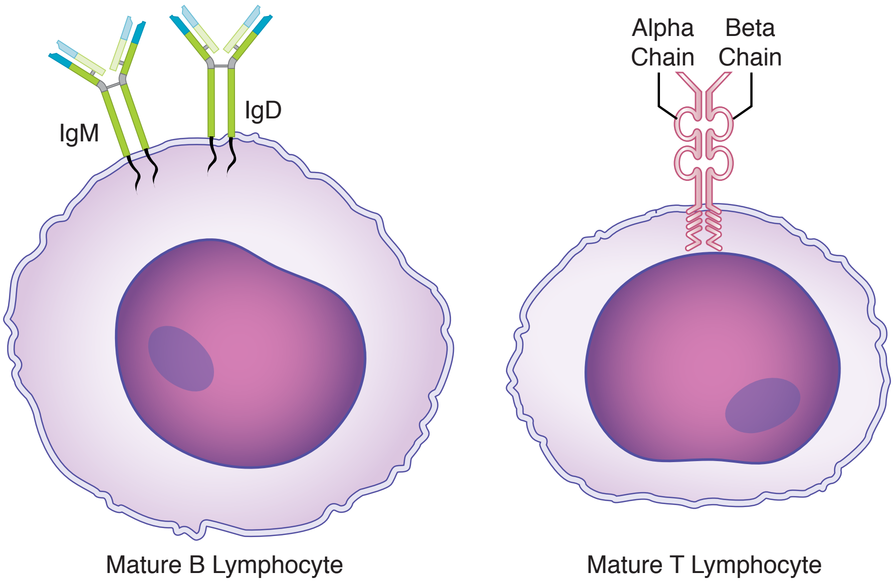


Figure I-3-1. Antigen Receptors of Mature Lymphocytes

The antigen receptor of the B lymphocyte, or **membrane-bound immunoglobulin**, is a 4-chain glycoprotein molecule that serves as the basic monomeric unit for each of the distinct antibody molecules destined to circulate freely in the serum. This monomer has 2 identical halves, each composed of a **heavy chain** and a **light chain**. A cytoplasmic tail on the carboxy-terminus of each heavy chain extends through the plasma membrane and anchors the molecule to the cell surface. The 2 halves are held together by disulfide bonds into a shape resembling a "Y." Some flexibility of movement is permitted between the halves by disulfide bonds forming a **hinge region**.

On the N-terminal end of the molecule where the heavy and light chains lie side by side, an antigen binding site is formed whose 3-dimensional shape will accommodate the noncovalent binding of one, or a very small number, of related antigens. The unique structure of the antigen binding site is called the **idiotype** of the molecule. Although 2 classes **(isotypes)** of membrane immunoglobulin (IgM and IgD) are coexpressed on the surface of a mature, naïve B lymphocyte, only one idiotype or **antigenic specificity** is expressed per cell (although in multiple copies). Each individual is capable of producing hundreds of millions of unique idiotypes.

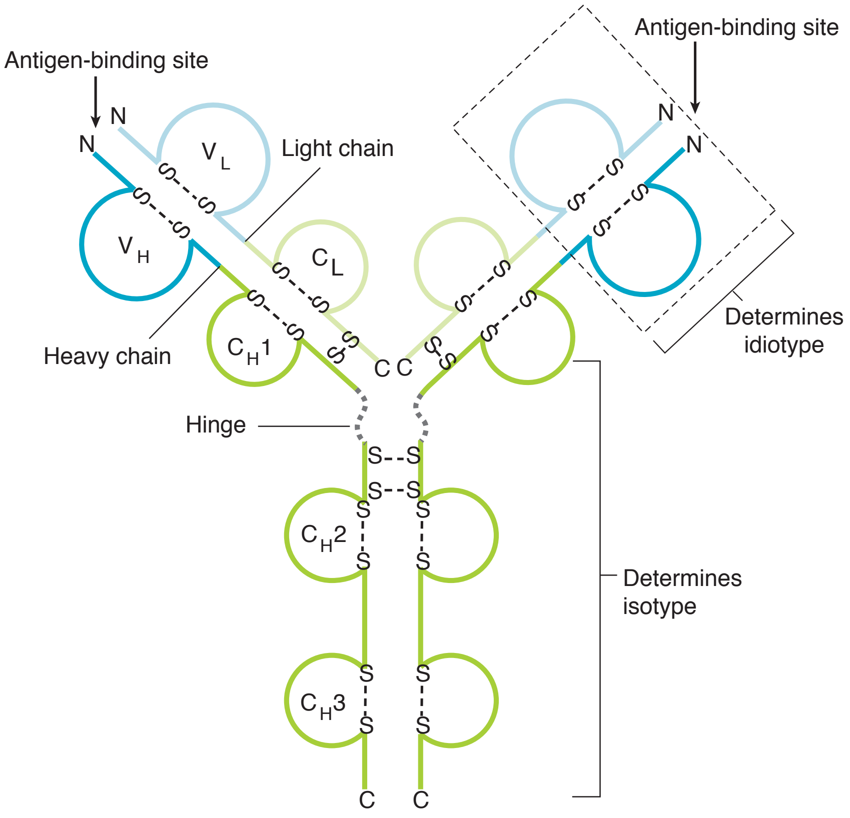


Figure I-3-2. B-Lymphocyte Antigen Recognition Molecule (Membrane-Bound Immunoglobulin)

The antigen receptor of the T lymphocyte is composed of 2 glycoprotein chains, a beta and alpha chain that are similar in length. On the carboxy-terminus of the chains, a cytoplasmic tail extends through the membrane for anchorage. On the N-terminal end of the molecule, an antigen-binding site is formed between the 2 chains, whose 3-dimensional shape will accommodate the binding of a small antigenic **peptide complexed to an MHC molecule** presented on the surface of an antigen-presenting cell. This groove forms the idiotype of the TCR. There is no hinge region present in this molecule, and thus its conformation is quite rigid.

The membrane receptors of B lymphocytes are designed to bind **unprocessed antigens** of almost any chemical composition, i.e., polysaccharides, proteins, lipids, whereas the TCR is designed to bind only **peptides complexed to MHC**. Also, although the B-cell receptor is ultimately modified to be secreted **antibody**, the TCR is never released from its membrane-bound location.

In association with these unique antigen-recognition molecules on the surface of B and T cells, accessory molecules are intimately associated with the receptors that function in signal transduction. Thus, when a lymphocyte binds to an antigen complementary to its idiotype, a cascade of messages transferred through its **signal transduction complex** will culminate in intracytoplasmic phosphorylation events leading to activation of the cell.

- In the B cell, this signal transduction complex is composed of 2 invariant chains, Ig-alpha and Ig-beta, and a B-cell co-receptor consisting of CD19, CD21 and CD81.
- The B-cell co-receptor is implicated in the attachment of several infectious agents. CD21 is the receptor for EBV and CD81 is the receptor for hepatitis C and *Plasmodium vivax*.
- In the T cell, the signal transduction complex is a multichain structure called CD3.

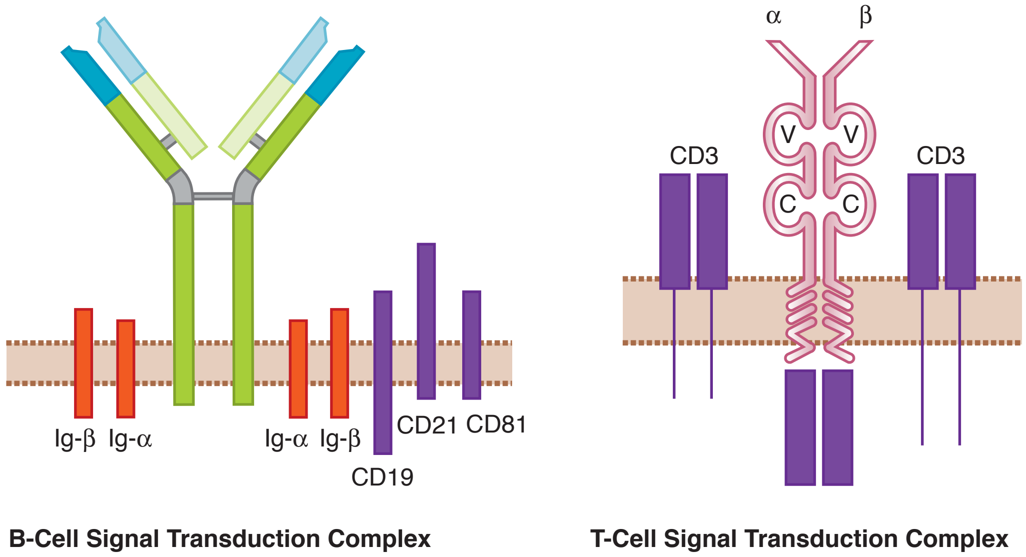


Figure I-3-3. Lymphocyte Signal Transduction

Table I-3-1. B- versus T-Lymphocyte Antigen Receptors

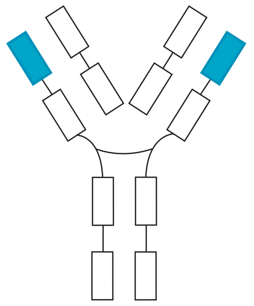
Property	B-Cell Antigen Receptor	T-Cell Antigen Receptor
Molecules/Lymphocyte	100,000	100,000
Idiotypes/Lymphocyte	1	1
Isotypes/Lymphocyte	2 (IgM and IgD)	1 (α/β)
Is secretion possible?	Yes	No
Number of combining sites/molecule	2	1
Mobility	Flexible (hinge region)	Rigid
Signal-transduction molecules	Ig-α, Ig-β, CD19, CD21	CD3

THE GENERATION OF RECEPTOR DIVERSITY

Because the body requires the ability to respond specifically to millions of potentially harmful agents it may encounter in a lifetime, a mechanism must exist to generate as many idiotypes of antigen receptors as necessary to meet this challenge. If each of these idiotypes was encoded separately in the germline DNA of lymphoid cells, it would require more DNA than is present in the entire cell. The generation of this necessary diversity is accomplished by a complex and unique set of rearrangements of DNA segments that takes place during the maturation of lymphoid cells.

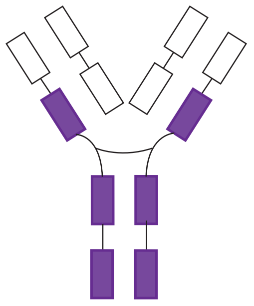
It has been discovered that individuals inherit a large number of different segments of DNA which may be recombined and alternatively spliced to create unique amino acid sequences in the N-terminal ends (**variable domains**) of the chains that compose their antigen recognition sites. For example, to produce the **heavy chain variable domains** of their antigen receptor, B-lymphocyte progenitors select randomly and in the absence of stimulating antigen to recombine 3 gene segments designated variable (V), diversity (D), and joining (J) out of hundreds of germline-encoded possibilities to produce unique sequences of amino acids in the variable domains (VDJ recombination).

An analogous random selection is made during the formation of the beta-chain of the TCR.



Note

VDJ rearrangements in DNA produce the diversity of heavy chain variable domains.



Note

mRNA molecules are created which join this variable domain sequence to μ or δ constant domains.

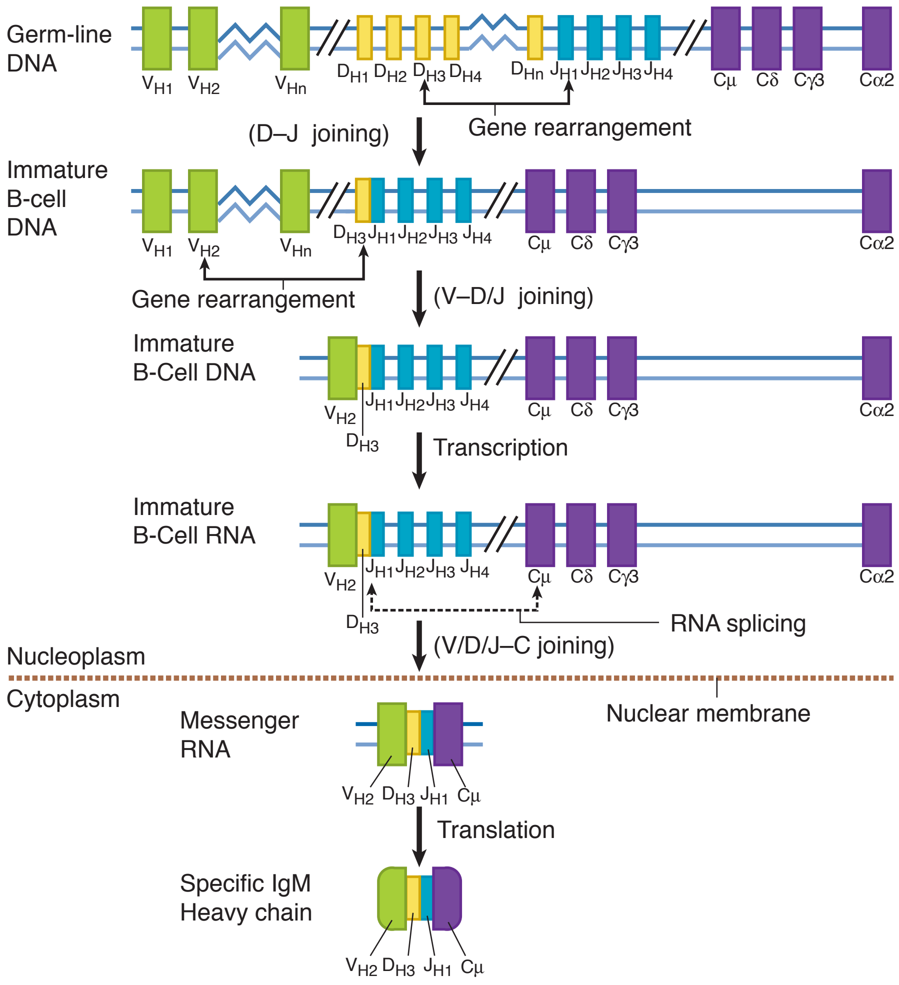


Figure I-3-4. Production of Heavy (B-Cell) or Beta (T-Cell) Chains of Lymphocyte Antigen Receptors

Next, the B-lymphocyte progenitor performs random rearrangements of 2 types of gene segments (V and J) to encode the **variable domain amino acids of the light chain.** An analogous random selection is made during the formation of the alpha-chain of the TCR. The enzymes responsible for these gene rearrangements are encoded by the genes *RAG1* and *RAG2*. The *RAG1* and *RAG2* gene products are 2 proteins found within the recombinase, a protein complex that includes a repair mechanism as well as DNA-modifying enzymes.

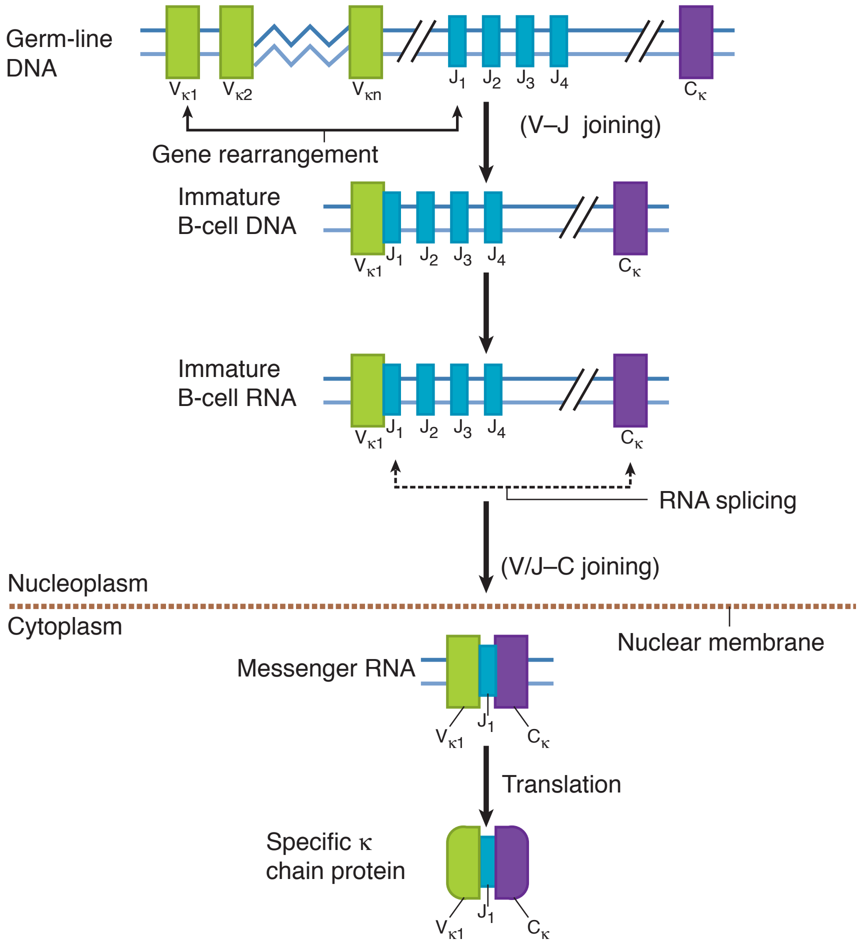
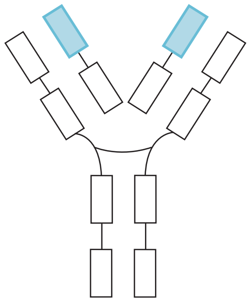


Figure I-3-5. Production of Light (B-Cell) or Alpha (T-Cell) Chain of a Lymphocyte Antigen Receptor

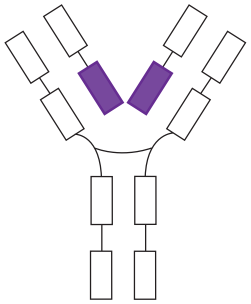
While heavy chain gene segments are undergoing recombination, the enzyme **terminal deoxyribonucleotidyl transferase** (Tdt) randomly inserts bases (without a template on the complementary strand) at the junctions of V, D, and J segments (**N-nucleotide addition**). The random addition of the nucleotide generates junctional diversity.

When the light chains are rearranged later, Tdt is not active, though it is active during the rearrangement of all gene segments in the formation of the TCR. This generates even more diversity than the random combination of V, D, and J segments alone.



Note

VJ rearrangements in DNA produce the diversity of light chain variable domains.



Note

K or λ constant domains are added to complete the light chain.

Bridge to Pathology

Tdt is used as a marker for early stage T- and B-cell development in acute lymphoblastic leukemia.

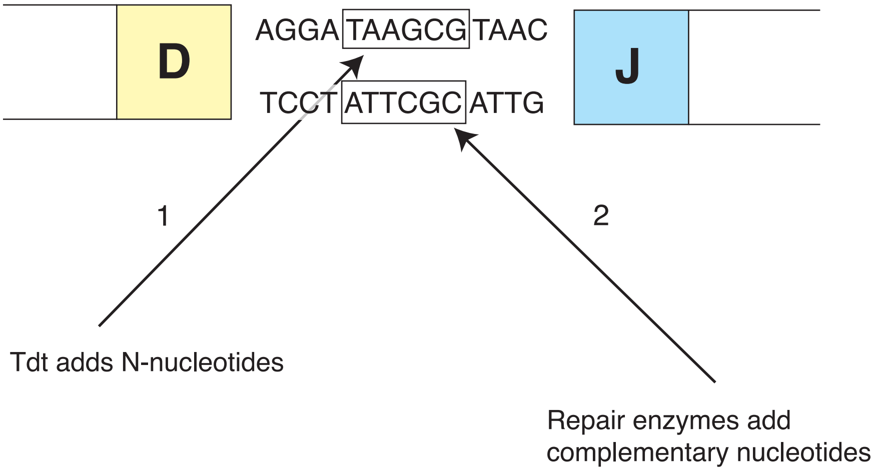


Figure I-3-6. Function of Tdt

Needless to say, many of these gene segment rearrangements result in the production of truncated or nonfunctional proteins. When this occurs, the cell has a second chance to produce a functional strand by rearranging the gene segments of the homologous chromosome. If it fails to make a functional protein from rearrangement of segments on either chromosome, the cell is induced to undergo **apoptosis** or programmed cell death.

In this way, the cell has 2 chances to produce a functional heavy (or β) chain. A similar process occurs with the light (or α) chain. Once a functional product has been achieved by one of these rearrangements, the cell shuts off the rearrangement and expression of the other allele on the homologous chromosome—a process known as **allelic exclusion**. This process ensures that B and T lymphocytes synthesize only **one specific antigen-receptor per cell**.

Because any heavy (or β) chain can associate with any randomly generated light (or α) chain, one can multiply the number of different possible heavy chains by the number of different possible light chains to yield the total number of possible idiotypes that can be formed. This generates yet another level of diversity.

Table I-3-2. Mechanisms for Generating Receptor Diversity

Mechanism	Cell in Which It Is Expressed
Existence in genome of multiple V, D, J segments	B and T cells
VDJ recombination	B and T cells
N-nucleotide addition	B cells (only heavy chain) T cells (all chains)
Combinatorial association of heavy and light chains	B and T cells
Somatic hypermutation	B cells only, after antigen stimulation (*see* Chapter 7)

Downstream on the germline DNA from the rearranged segments, are encoded the amino acid sequences of all the constant domains of the chain. These domains tend to be similar within the classes or isotypes of immunoglobulin or TCR chains and are thus called **constant domains**.

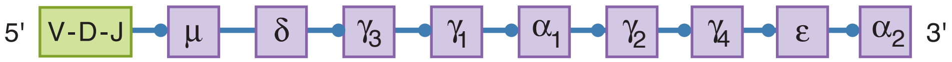


Figure I-3-7. Immunoglobulin Heavy Chain DNA

The first set of constant domains for the heavy chain of immunoglobulin that is transcribed is that of IgM and next, IgD. These 2 sets of domains are alternatively spliced to the variable domain product at the RNA level. There are only 2 isotypes of light chain constant domains, named κ and λ, and one will be combined with the product of light chain variable domain rearrangement to produce the other half of the final molecule. Thus, the B lymphocyte produces IgM and IgD molecules with identical idiotypes and inserts these into the membrane for antigen recognition.

Table I-3-3. Clinical Outcomes of Failed Gene Rearrangement

Clinical Syndrome	Genetics	Molecular Defect	Symptoms
Omenn syndrome	Autosomal recessive	Missense mutation in *RAG* genes The *RAG* enzymes have only partial activity	Lack of B cells (below limits of detection) Marked decrease in predominantly Th2 Characterized by early onset, failure to thrive, red rash (generalized), diarrhea, and severe immune deficiency
Severe combined immunodeficiency (SCID)	Autosomal recessive	Null mutations in *RAG1* or *RAG2* genes No *RAG* enzyme activity	Total lack of B and T cells Total defects in humoral and cell-mediated immunity

SELECTION OF T AND B LYMPHOCYTES

As lymphoid progenitors develop in the bone marrow, they make random rearrangements of their germline DNA to produce the unique idiotypes of antigen-recognition molecules that they will use throughout their lives. The bone marrow, therefore, is considered a **primary lymphoid organ** in humans because it supports and encourages these early developmental changes. B lymphocytes complete their entire formative period in the bone marrow and can be identified in their progress by the immunoglobulin chains they produce.

B Lymphocyte Development

In essence, the rearrangement of the gene segments and the subsequent production of immunoglobulin chains drive B-cell development.

Because these gene segment rearrangements occur randomly and in the absence of stimulation with foreign antigen, it stands to reason that many of the idiotypes of receptors produced could have a binding attraction or **affinity** for normal body

constituents. These cells, if allowed to develop further, could develop into self-reactive lymphocytes that could cause harm to the host. Therefore, one of the key roles of the bone marrow stroma and interdigitating cells is to remove such potentially harmful products. Cells whose idiotype has too great an affinity for normal cellular molecules are either deleted in the bone marrow (**clonal deletion**) or inactivated in the periphery (**clonal anergy**). Anergic B cells express high levels of IgD on their surface rendering them inactive. The elimination of self-reactive cells in the bone marrow is intended to minimize the number of self-reactive B-lymphocytes released to the periphery, only those cells that are **selectively unresponsive** (**tolerant**) to self-antigens are allowed to leave the bone marrow.

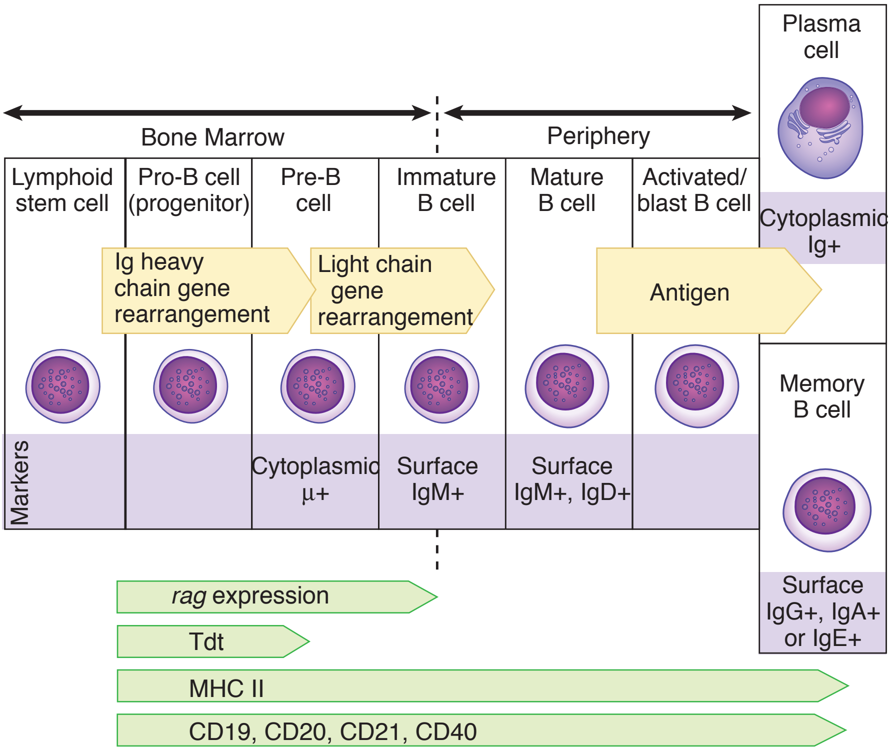


Figure I-3-8. B-Cell Differentiation

T Lymphocyte Development

Immature lymphocytes destined to the T-cell lineage leave the bone marrow and proceed to the **thymus**, the second **primary lymphoid organ** dedicated to the maturation of T cells. These pre-thymic cells are referred to as **double negative** T lymphocytes since they do not express CD4 or CD8 on their surface. The thymus is a bilobed structure located above the heart; it consists of an outer **cortex** packed with immature T cells and an inner **medulla** into which cells pass as they mature. Both the cortex and medulla are laced with a network of epithelial cells, dendritic cells, and macrophages, which interact physically with the developing thymocytes.

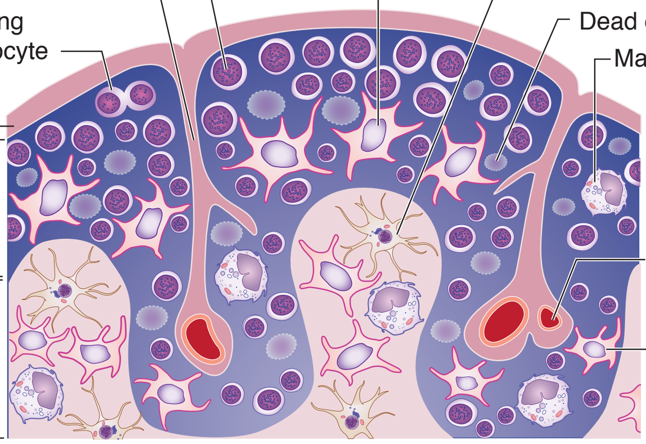


Figure I-3-9. Structure of the Thymus

Within the cortex, the thymocytes will begin to rearrange the beta and alpha chains of the T-cell receptor (TCR) while coexpressing the CD3 complex as well as the CD4 and CD8 co-receptors; these thymocytes are collectively referred to as being **double positive**. As the developing thymocytes begin to express their TCRs, they are subjected to a rigorous 2-step selection process. Because the TCR is designed to bind antigenic peptides presented on the surface of **antigen-presenting cells** (APCs) in the body, a selection process is necessary to remove those cells that would bind to normal self-antigens and cause **autoimmunity,** as well as those that have no attraction whatsoever for the surfaces of APCs. This is accomplished by exposure of developing thymocytes to high levels of a unique group of membrane-bound molecules known as **major histocompatibility complex** (MHC) antigens.

The MHC is a collection of highly polymorphic genes on the short arm of chromosome 6 in the human. There are 2 major classes of cell-bound MHC gene products: I and II. Both class I and class II molecules are expressed at high density on the surface of cells of the thymic stroma. MHC gene products are also called human leukocyte antigens (HLA).

- **Class I** MHC gene products: HLA-A, HLA-B, HLA-C
- **Class II** MHC gene products: HLA-DM, HLA-DP, HLA-DQ, HLA-DR

Table I-3-4. Class I and II Gene Products

Class I Gene Products			Class II Gene Products			
HLA-A	HLA-B	HLA-C	HLA-DM*	HLA-DP	HLA-DQ	HLA-DR

*HLA-DM is not a cell surface molecule but functions as a molecular chaperone to promote proper peptide loading.

Class I molecules are expressed on all nucleated cells in the body, as well as platelets. They are expressed in **codominant** fashion, meaning that each cell expresses 2 A, 2 B, and 2 C products (one from each parent).

- The molecules (A, B, and C) consist of an α heavy chain with 3 extracellular domains and an intracytoplasmic carboxy-terminus.
- A second light chain, β_2-microglobulin, is not encoded within the MHC and functions in peptide-loading and transport of the class I antigen to the cell surface.
- A groove between the first 2 extracellular domains of the α chain is designed to accommodate small peptides to be presented to the TCR.

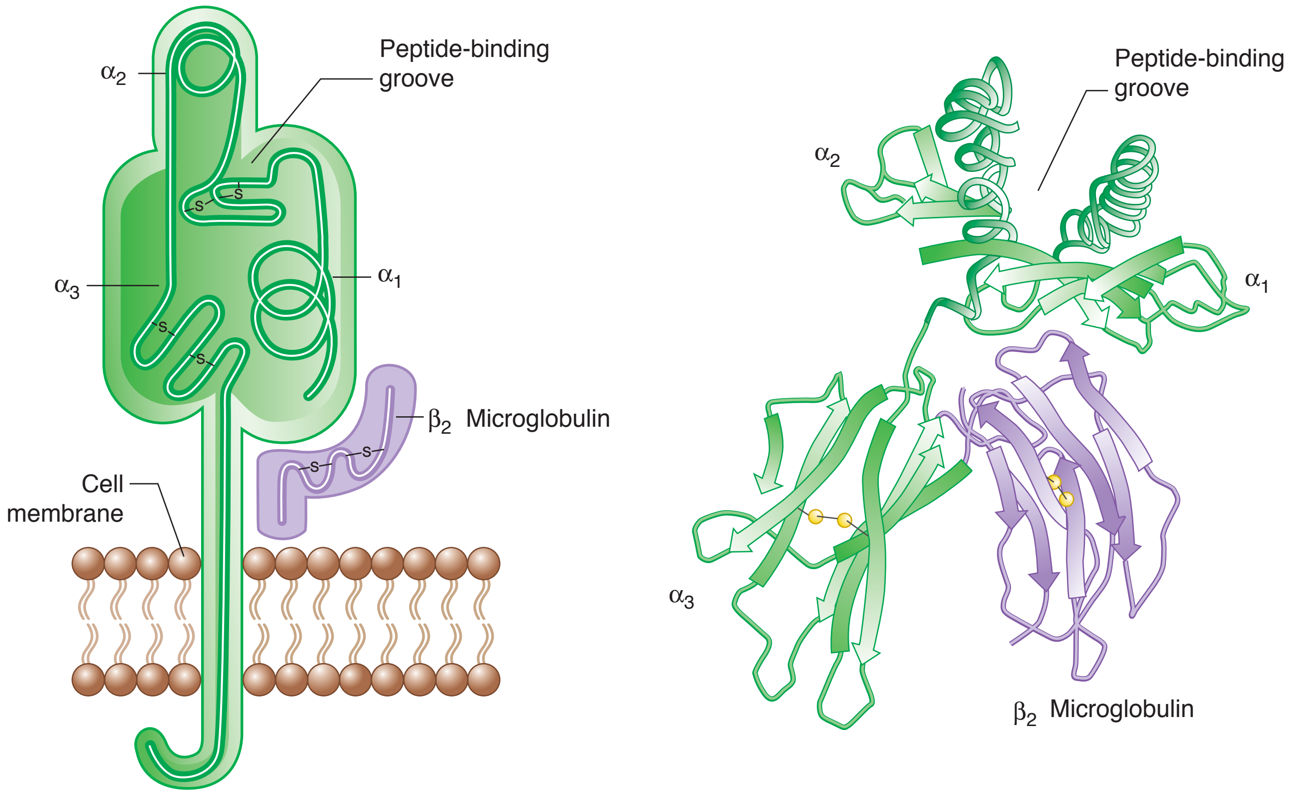


Figure I-3-10. Class I MHC Molecule (left) and X-Ray Crystallographic Image (right) of Class I MHC Peptide-Binding Groove

Class II MHC molecules are expressed (also **codominantly**) on the professional antigen-presenting cells of the body (primarily the macrophages, B lymphocytes, and dendritic cells).

- The molecules are 2 chain structures of similar length, called α and β, each possessing 2 extracellular domains and 1 intracytoplasmic domain.
- A groove that will accommodate peptides to be presented to the TCR is formed at the N-terminal end of both chains.

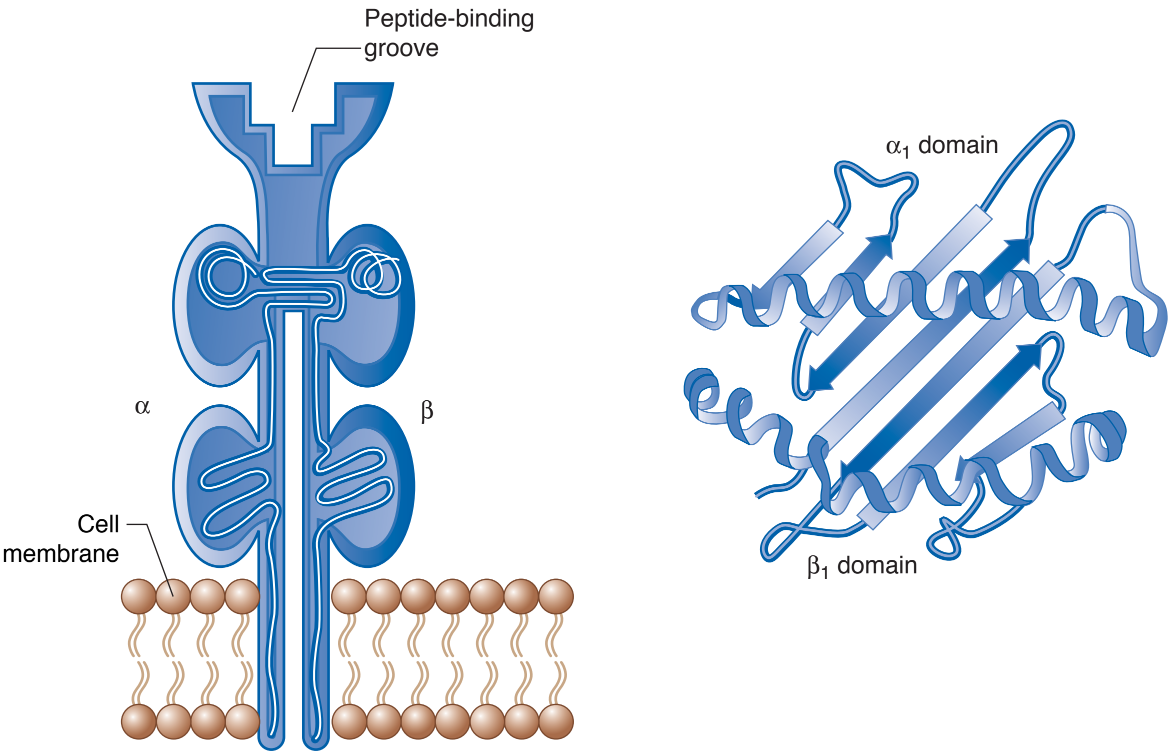


Figure I-3-11. Class II MHC Molecule (left), and X-ray Crystallographic Image (right) of Class II MHC Peptide-Binding Groove

Within the thymus, each of these MHC products, loaded with normal self-peptides, is presented to the developing double positive thymocytes.

- Those that have TCRs capable of binding with low affinity will receive a **positive selection** signal to divide and establish clones that will eventually mature in the medulla.
- Those that fail to recognize self-MHC at all will not be encouraged to mature (**failure of positive selection**).
- Those that bind too strongly to self MHC molecules and self-peptide will be induced to undergo apoptosis (**negative selection**) because these cells would have the potential to cause autoimmune disease.

Although double positive thymocytes co-express **CD4** and **CD8**, the cells are directed to express only CD8 if their TCR binds class I molecules, and only CD4 if their TCR binds class II molecules. At this point in T-cell development, the thymocytes are referred to as being **single positive**.

This selection process is an extraordinarily rigorous one. A total of 95–99% of all T-cell precursors entering the thymus are destined to die there. Only those with TCRs appropriate to protect the host from foreign invaders will be permitted to exit to the periphery: CD4+ cells that recognize class II MHC are destined to become **"helper" T cells** (Th), and CD8+ cells that recognize class I MHC are destined to become **cytotoxic T lymphocytes** (CTLs).

While most self-reactive T cells will be deleted in the thymus, a small population of these T cells will instead differentiate into regulatory T cells (Tregs). Tregs inhibit self-reactive Th1 cells in the periphery.

- Identified by their constitutive expression of CD25 on the surface and by the expression of the transcription factor FoxP3
- Secrete IL-10 and TGF-β which inhibit inflammation
- Shown to be critical in the prevention of autoimmunity

Tregs will leave the thymus and serve in a peripheral tolerance.

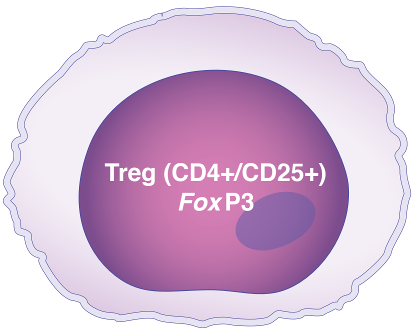


Figure I-3-12. Identification of Treg Cells

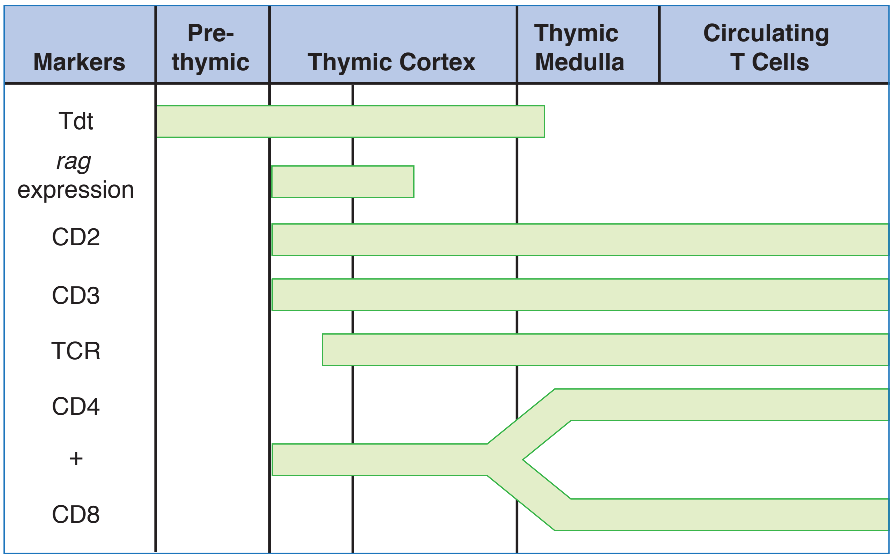


Figure I-3-13. Human T-Cell Differentiation

Periphery: Innate Immune Response 4

Learning Objectives

- ❑ Describe the structure and function of secondary lymphoid organs
- ❑ Describe the structure of lymph nodes
- ❑ Answer questions about chemokines and adhesion molecules

INNATE IMMUNITY

The innate immune system is an important part of any immune response. It is responsible for reacting quickly to invading microbes and for keeping the host alive while the adaptive immune system is developing a very specific response. The innate immune defenses are all present at birth; they have a very limited diversity for antigen, and they attack the microbes with the same basic vigor no matter how many times they have seen the same pathogen.

The innate immune system handles pathogens in 2 general ways:

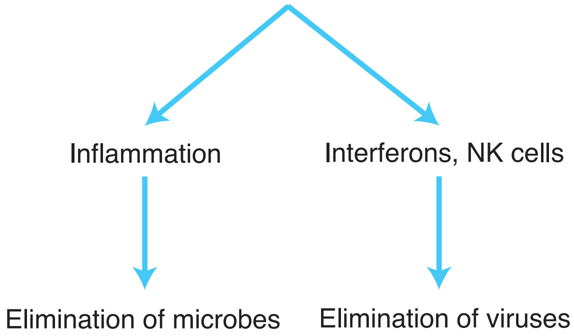


Figure I-4-1. Pathogen Clearance by the Innate Immune System

Microbes may gain access to the tissues if the physical barriers are breached. In the tissues, they come in contact with phagocytic cells such as neutrophils, macrophages and dendritic cells, which will produce chemical messengers called **cytokines** that can initiate an inflammatory response.

Many times the innate immune components are enough to eliminate the pathogen, but not always. The pathogens may gain access to the blood, in which the alternate pathway for complement activation may provide some additional help. But this is where the adaptive immune system may have to take over to resolve the infection and eliminate the pathogen.

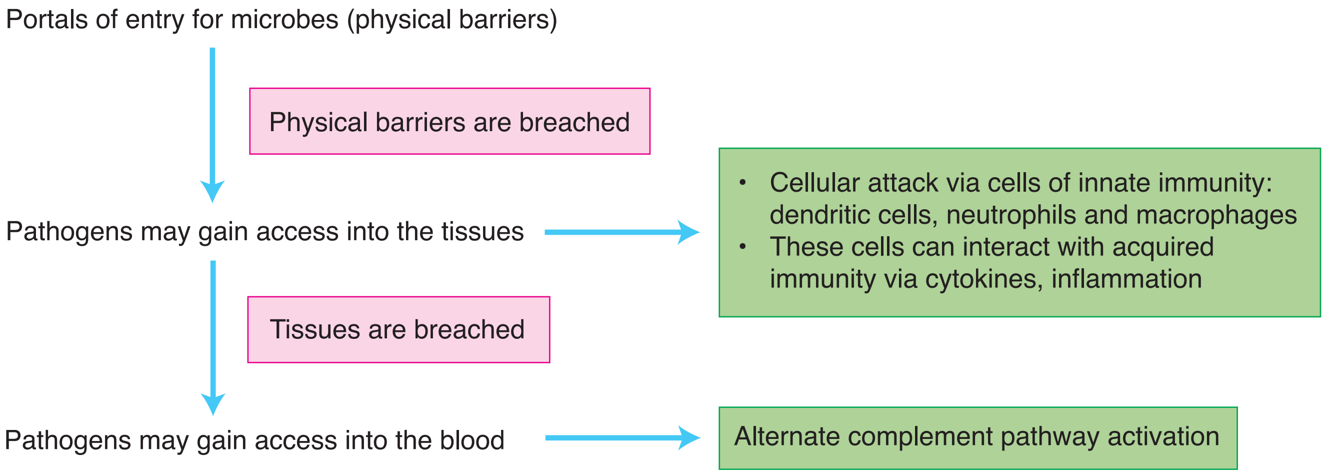


Figure I-4-2. Entry Sites for Pathogens

INNATE IMMUNE COMPONENTS/BARRIERS

There are several components to the innate immune response that are essential for this early defense against pathogens. They will be introduced here and discussed in more depth later in this chapter. They include **physical (anatomic) barriers, physiologic barriers, innate cellular response**, and **inflammation**.

Anatomic Barriers

The main portals of entry for most pathogens are the skin, the respiratory tract, and the GI tract. All of these surfaces are lined with epithelial cells that can produce a few antimicrobial products such as defensins and interferons. They may also contain a number of specialized intra-epithelial lymphocytes (IEL) called γδ T cells. These specialized T cells are considered part of innate immunity as they can only recognize shared microbial structures.

- The skin is a great physical barrier as most pathogens can't invade intact skin. The pH of the skin is also slightly acidic and can retard the growth of pathogenic organisms.
- The respiratory tract is lined with cilia that physically attempt to remove microbes as they enter. Saliva and mucous are also difficult environments for microbes to live in, as there are many antimicrobial enzymes and chemicals within those entities.
- The GI tract is also a mucous membrane with similar properties to the respiratory tract; however, pathogens that enter here must first survive a trip through the stomach with a highly acidic pH that kills many microorganisms.

Physiologic Barriers

Physiologic barriers include the following components:

- **Temperature**
  - Many microbial pathogens can't survive much past human body temperature. When the inflammatory response is initiated in the local tissues, cytokines may act systemically to alter the temperature set point in the hypothalamus resulting in fever.
- **pH**
  - The acidic pH of the stomach impedes the growth and transmission to the gut of many pathogens.
  - The skin is also acidic and retards the growth of many microorganisms.
- **Chemical**
  - Lysozyme present in secretions such as tears, saliva, breast milk and mucous can break down the cell wall peptidoglycan of bacteria.
  - Defensins found within phagocytes can form pores in bacteria and fungi.
- **Interferons**
  - IFN-α and IFN-β are anti-viral interferons. They have a direct anti-viral effect by transiently inhibiting nascent protein synthesis in cells.

Innate Cellular Response

Phagocytic cells (monocytes/macrophages, neutrophils and dendritic cells) are part of the first line of defense against invading pathogens. They recognize pathogens via shared molecules that are not expressed on host cells. They are responsible for controlling the infections and sometimes are even capable of eradicating them.

Receptors of the innate immune system are referred to as **pattern recognition receptors (PRRs)**. PRRs recognize **pathogen-associated molecular patterns (PAMPs),** molecules that are shared by pathogens of the same type (bacterial LPS, n-formyl peptides etc.) or **damage-associated molecular patterns (DAMPs)** released from dying or damaged cells. These receptors are present intrinsically, encoded in the germline genes, and are not generated through somatic recombination as the lymphocyte receptors are generated.

The innate immune system can recognize <1,000 patterns on various pathogens, compared to the adaptive immune system (B and T cells) which can recognize over 1 billion specific sequences on pathogens.

Inflammasome

The inflammasome is an important part of the innate immune system. It is expressed in myeloid cells as a signalling system for detection of pathogens and stressors. Activation of the inflammasome results in the production of IL-1β and IL-18, which are potent inflammatory cytokines.

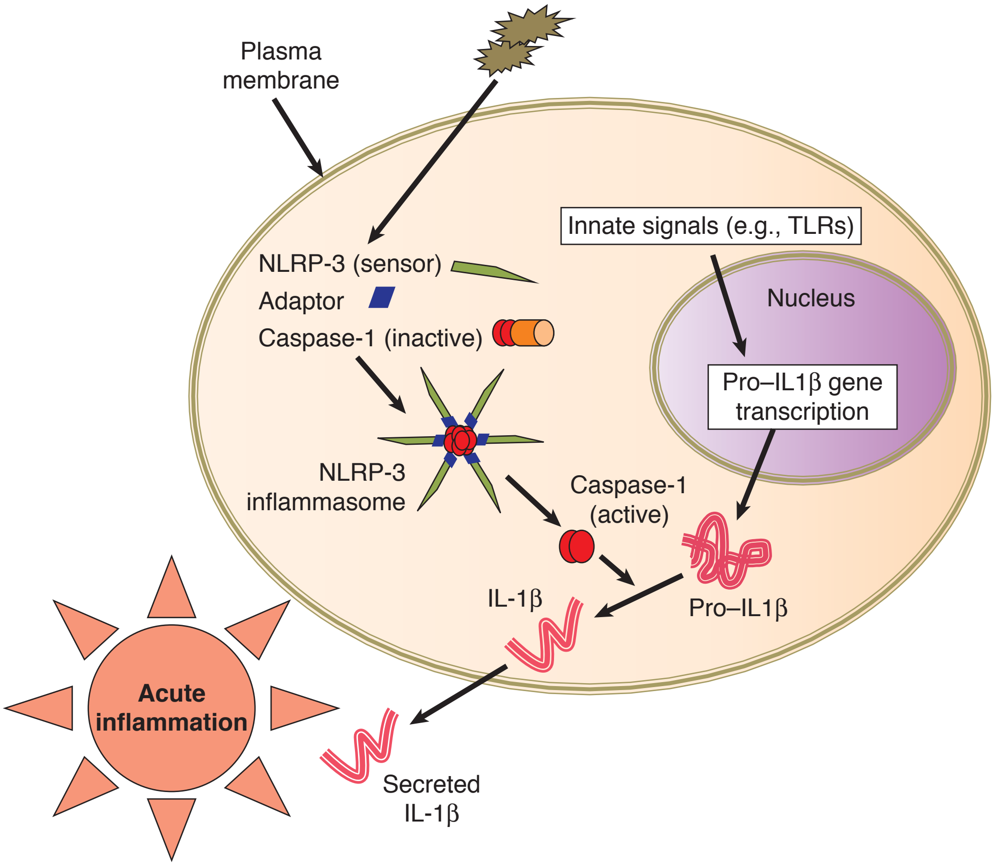


Figure I-4-3. Inflammasome

Table I-4-1. Receptors of the Innate Immune System

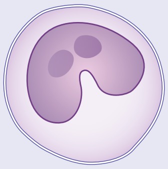
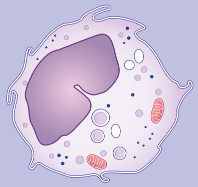
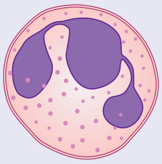
Receptor Type	Location in Cell	Receptor Name	Pathogen Target	Downstream Effects
Toll-like receptors (TLR)	Extracellular	TLR-1, 2, 6	Bacterial lipopeptides	Activation of transcription factors (including NF-κB) which results in the transcription of cytokines, adhesion molecules, and enzymes that are antimicrobial
		TLR-2	Bacterial peptidoglycan	
		TLR-4	Lipopolysaccharide (LPS)	
		TLR-5	Flagellin	
	Intracellular (endosomal)	TLR-3	DS RNA	
		TLR-7,8	SS RNA	
		TLR-9	Unmethylated CpG oligonucleotides	
NOD-like receptors (NLR)	Intracellular (cytosolic)	NOD1, NOD2	Components of bacterial PG	Signals via NF-κB result in macrophage activation
		NLRP-3	Microbial products and molecules from damaged or dying cells (ATP, uric acid crystals, reactive oxygen species)	Inflammasome NLRP-3 (sensor) + adaptor protein links procaspase 1 and activates it to caspase 1; it is the caspase that cleaves the pro-IL-1β to generate IL-1β
RIG-like receptors (RLR)	Cytoplasmic	RIG-1, MDA-5	Viral RNA	Interferon production

Clinical Correlate

Mutations in Innate Immune Receptors and Correlation with Disease

Mutation	Effect
Mutation in signaling molecules effecting TLRs	Recurrent, severe bacterial infections (pneumonia)
Gain of function mutations in inflammasome	• Gout • Atherosclerosis • Type II diabetes
NOD-2 mutations	IBD
• IL-12 receptor deficiency • IFN-γ receptor deficiency	Recurrent infections with intracellular bacteria (*Mycobacterium*)

Table I-4-2. Myeloid Cells

Myeloid Cell	Tissue Location	Identification	Function
Monocyte 	Bloodstream, 0–900/µL	Kidney bean-shaped nucleus, CD14 positive	Phagocytic, differentiate into tissue macrophages
Macrophage 	Tissues	Ruffled membrane, cytoplasm with vacuoles and vesicles, CD14 positive	Phagocytosis, secretion of cytokines
Neutrophil 	Bloodstream, 1,800–7,800/µL	Multilobed nucleus; small light pink to purple granules CD14 positive	Phagocytosis and activation of bactericidal mechanisms

Cells of Innate Immunity

Neutrophils

- Circulating phagocytes
- Short lived
- Rapid response, not prolonged defense

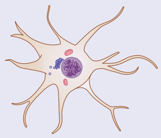
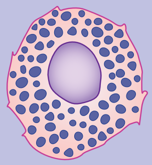
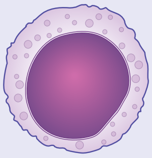
Monocytes/Macrophages

- Monocytes circulate in the blood, become macrophages in the tissues
- Provide a prolonged defense
- Produce cytokines that initiate and regulate inflammation
- Phagocytose pathogens
- Clear dead tissue and initiate tissue repair
- Macrophages will develop along one of 2 different pathways

Table I-4-3. Pathways for Macrophage Activation

Classical M1	Alternative M2
Induced by innate immunity (TLRs, IFN-γ)	Induced by IL-4, IL-13
Phagocytosis, initiate inflammatory response	Tissue repair and control of inflammation

Table I-4-4. Additional Myeloid Cells

Myeloid Cell	Tissue Location	Identification	Function
Dendritic Cells 	Epithelia, tissues	Long cytoplasmic arms CD14 positive	Antigen capture, transport, and presentation, initiate inflammation
Mast Cells 	Tissues, mucosa, epithelia	Small nucleus, cytoplasm packed with large blue granules	Release of granules containing histamine, etc., during allergic responses
Natural killer Lymphocyte 	Lymph nodes, spleen, mucosal-associated lymphoid tissues, bone marrow	Lymphocytes with large cytoplasmic granules CD16 + CD56 positive	Kill tumor/virus cell targets or antibody-coated target cells, secretion of IFN-γ

Dendritic cells (DCs)

- Found in all tissues
- Antigen processing and presentation
- Two major functions: initiate inflammatory response and stimulate adaptive immune response

Mast cells

- Skin, mucosa
- 2 pathways for activation: innate TLRs and antibody-dependent (IgE)

Natural killer cells (NK cells)

- Blood, periphery
- Direct lysis of cells, secretion of IFN-γ

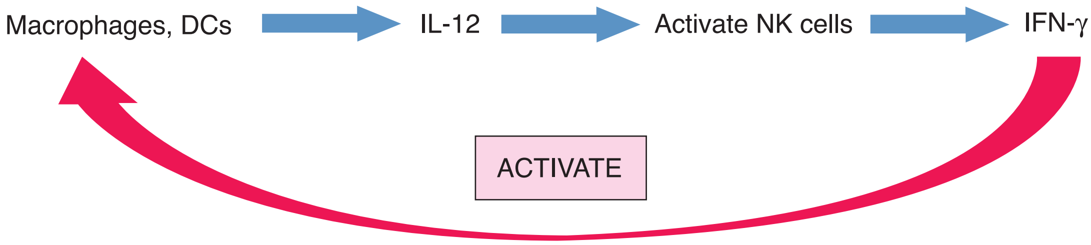


Figure I-4-4. Collaboration of Macrophages and NK Cells

INFLAMMATORY RESPONSE

Complement

The complement system is a set of interacting proteins released into the blood after production in the liver. The components act together as zymogens, activating one another in cascade fashion after initiation from a variety of stimuli. Three pathways of activation occur in the body and culminate similarly in the production of important split products that mediate inflammation, enhance phagocytosis by opsonization, and cause lysis of particles by membrane pore formation.

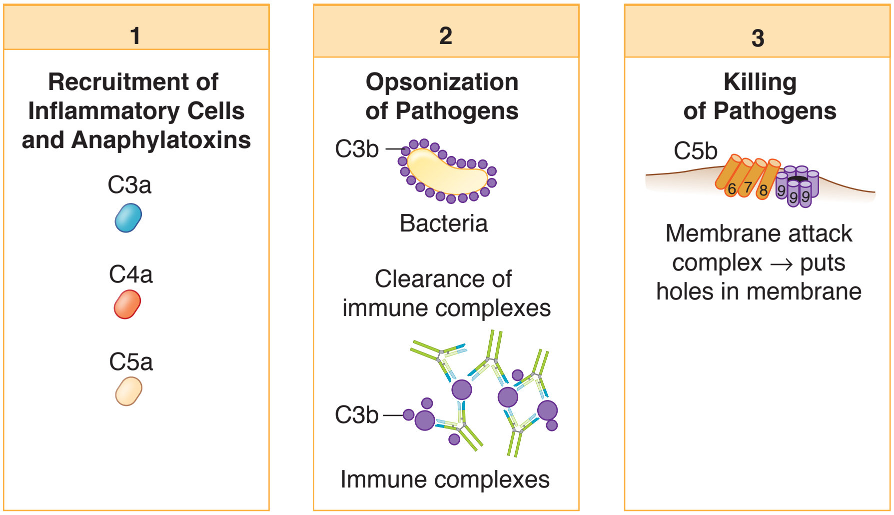


Figure I-4-5. Three Functions of the Complement System

Two of the pathways are considered part of the innate immune system: the alternate pathway and the lectin-binding or mannose-binding pathway (MBP). The alternate pathway for complement activation is shown below; the MBP activates the classical complement pathway but without the use of antibody, and is therefore considered part of innate immunity. The MBP is activated when mannose-binding lectin binds to carbohydrates on the pathogen.

The alternative pathway of complement activation is probably the more primitive of the pathways because it is initiated by simple attraction of the early factors to the surfaces of microbes. Bacterial polysaccharides and the lipopolysaccharide of the cell envelope of gram-negative bacteria both serve as potent, initiating stimuli.

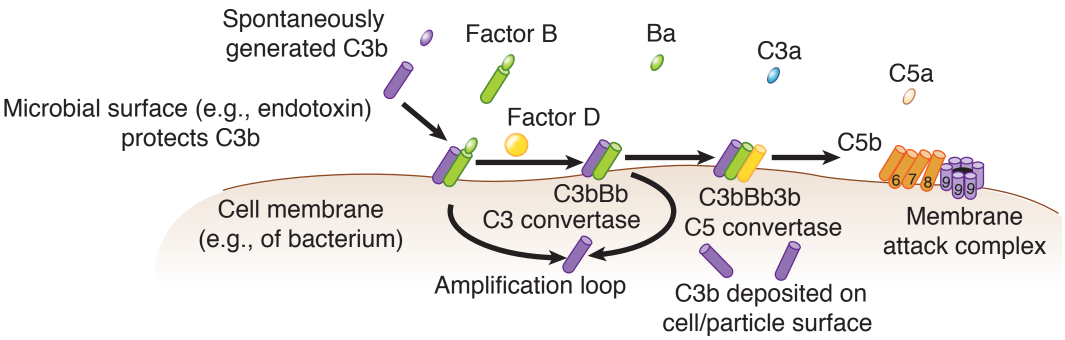


Figure I-4-6. The Alternative Complement Pathway

Acute Inflammatory Response

Antigens are normally introduced into the body across the mucosa or the epithelia. The **acute inflammatory response** is often the first response to this invasion and represents a response of the innate immune system to block the challenge.

The first step in the acute inflammatory response is activation of the vascular endothelium in the breached epithelial barrier. Cytokines and other inflammatory mediators released in the area as a result of tissue damage induce expression of selectin-type adhesion molecules on the endothelial cells. Neutrophils are the first cells to bind to the inflamed endothelium and extravasate into the tissues, peaking within 6 hours. Monocytes, macrophages, and even eosinophils may arrive 5–6 hours later in response to neutrophil-released mediators.

The extravasation of phagocytes into the area requires **4 sequential, overlapping steps**:

Step 1: Rolling

Phagocytes attach loosely to the endothelium by low-affinity, selectin-carbohydrate interactions. E-selectin molecules on the endothelium bind to mucin-like adhesion molecules on the phagocyte membrane and bind the cell briefly, but the force of blood flow into the area causes the cell to detach and reattach repeatedly, rolling along the endothelial surface until stronger binding forces can be elicited.

Step 2: Activation by chemo-attractants

Chemokines released in the area during inflammation, such as interleukin 8 (IL-8), complement split product C5a, and N-formyl peptides produced by bacteria bind to receptors on the phagocyte surface and trigger a G-protein–mediated activating signal. This signal induces a conformational change in integrin molecules in the phagocyte membrane that increases their affinity for immunoglobulin-superfamily adhesion molecules on the endothelium.

Step 3: Arrest and adhesion

Interaction between integrins and Ig-superfamily cellular adhesion molecules (Ig-CAMs) mediates the tight binding of the phagocyte to the endothelial cell. These integrin-IgCAM interactions also mediate the tight binding of phagocytes and their movement through the extracellular matrix.

Step 4: Transendothelial migration

The phagocyte extends pseudopodia through the vessel wall and extravasates into the tissues.

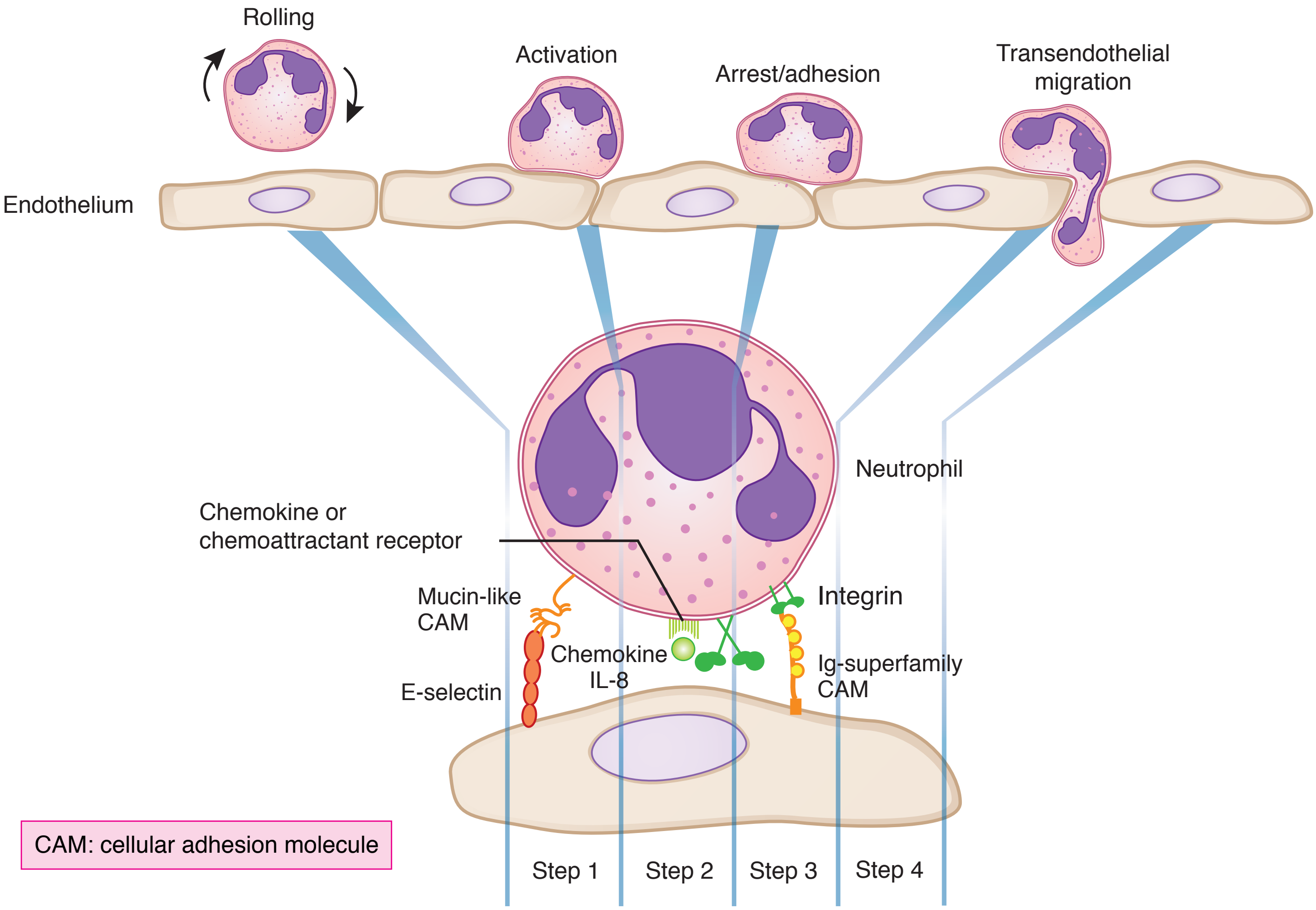


Figure I-4-7. Steps of Phagocyte Extravasation

Clinical Correlate

Leukocyte adhesion deficiency (LAD) is a rare autosomal recessive disease in which there is an absence of **CD18** (the common β_2 chain of a number of integrin molecules). A key element in the migration of leukocytes is integrin-mediated cell adhesion; patients suffer from an inability of their leukocytes to undergo adhesion-dependent migration into sites of inflammation. The first indication of this defect is often omphalitis, a swelling and reddening around the stalk of the umbilical cord.

- Patients are no more susceptible to viral infection than are normal controls, but they suffer recurrent, chronic bacterial infections.
- Patients frequently have abnormally high numbers of granulocytes in their circulation, but migration into sites of infection is not possible, so **abscess and pus formation do not occur**.
- One method for diagnosing LAD involves evaluating expression (or lack) of the β chain (CD18) of the integrin by flow cytometry.
- Bacterial infections in LAD patients can be treated with antibiotics but they recur. If a suitable bone marrow donor can be found, the hematopoietic system of the patient is destroyed with cytotoxic chemicals and a bone marrow transplant is performed.

Once in the tissues, neutrophils express increased levels of receptors for chemoattractants and exhibit chemotaxis migrating up a concentration gradient toward the attractant. Neutrophils release chemoattractive factors that call in other phagocytes.

Table I-4-5. Chemoattractive Molecules

Chemoattractive Molecule	Origin
Chemokines (IL-8)	Tissue mast cells, platelets, neutrophils, **monocytes**, **macrophages**, eosinophils, basophils, lymphocytes
Complement split product C5a	Classical or alternative pathways
Leukotriene B_4	Membrane phospholipids of macrophages, monocytes, neutrophils, mast cells → arachidonic acid cascade → lipoxygenase pathway
Formyl methionyl peptides	Released from microorganisms

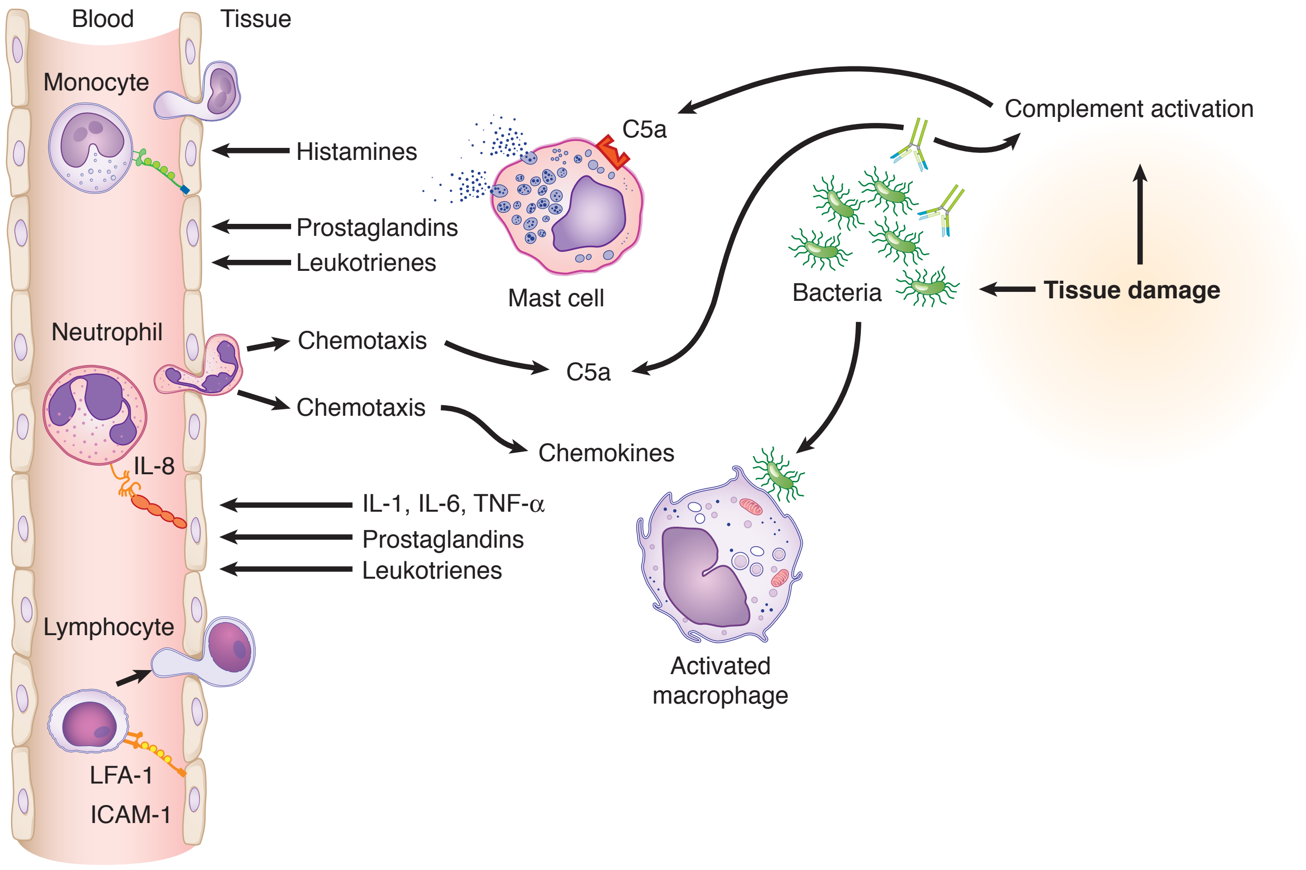


Figure I-4-8. Acute Inflammatory Response

Phagocytosis

Once chemotaxis of phagocytic cells into the area of antigen entry is accomplished, these cells ingest and digest particulate debris, such as microorganisms, host cellular debris, and activated clotting factors. This process, called phagocytosis, involves the following:

- Extension of pseudopodia to engulf attached material
- Fusion of the pseudopodia to trap the material in a phagosome
- Fusion of the phagosome with a lysosome to create a phagolysosome
- Digestion
- Exocytosis of digested contents

Neutrophils release granule contents into extracellular milieu during phagocytosis and inflammation in which the neutrophils die, forming what is known as pus. They extrude nuclear contents, histones, neutrophil extracellular traps (NETs) which function to:

- Trap and kill pathogens
- May damage tissues when enzymes, ROS get released into tissues

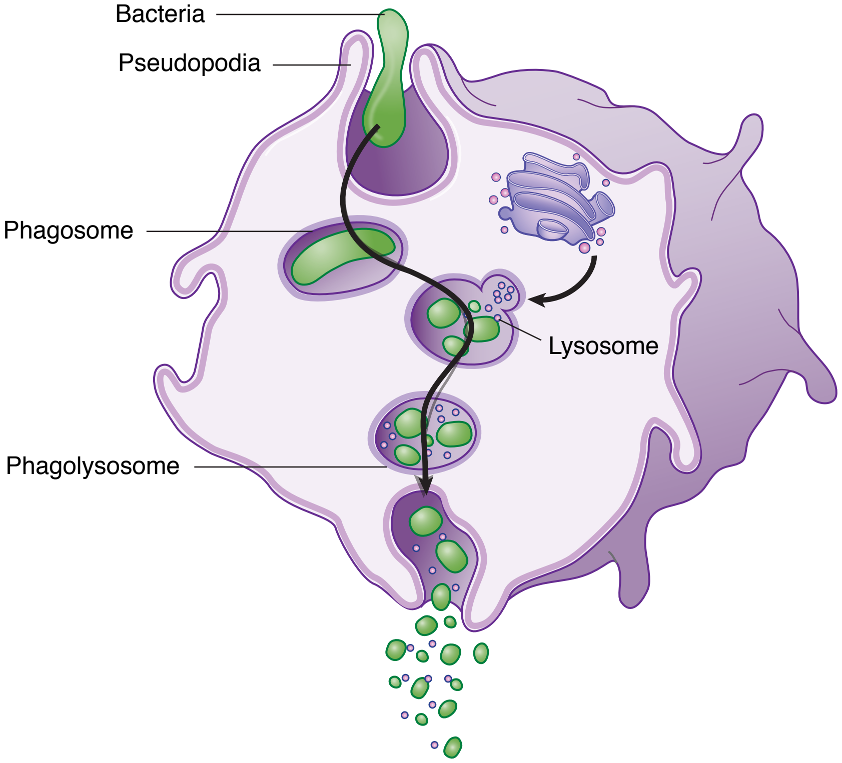


Figure I-4-9. Phagocytosis

Opsonization

Both macrophages and neutrophils have membrane receptors for certain types of antibody (IgG) and certain complement components (C3b). If an antigen is coated with either of these materials, adherence and phagocytosis may be enhanced by up to 4,000-fold. Thus, antibody and complement are called **opsonins**, and the means by which they enhance phagocytosis is called opsonization.

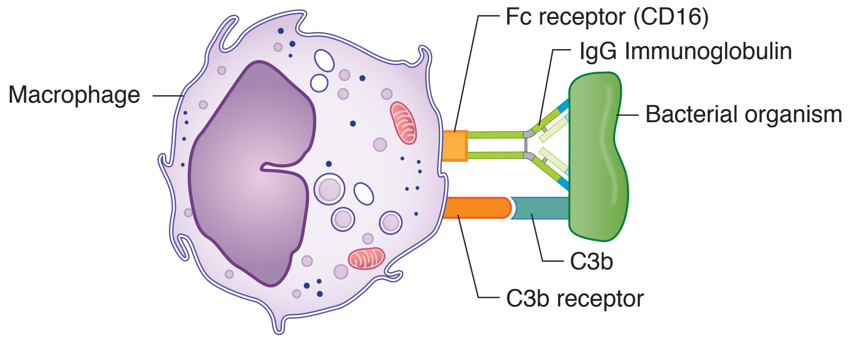


Figure I-4-10. Opsonization of Bacteria with Antibody and Complement C3b

Intracellular Killing

During phagocytosis, a metabolic process known as the respiratory burst activates a membrane-bound oxidase that generates oxygen metabolites, which are toxic to ingested microorganisms. Two oxygen-dependent mechanisms of intracellular digestion are activated as a result of this process.

- NADPH oxidase reduces oxygen to superoxide anion, which generates hydroxyl radicals and hydrogen peroxide, which are microbicidal.
- Myeloperoxidase in the lysosomes acts on hydrogen peroxide and chloride ions to produce hypochlorite (the active ingredient in household bleach), which is microbicidal.

Additionally, reactive nitrogen intermediates play an important role. Inducible nitric oxide synthase converts arginine to nitric oxide, which has potent antimicrobial properties.

The lysosomal contents of phagocytes contain oxygen-independent degradative materials:

- Lysozyme digests bacterial cell walls by cleaving peptidoglycan
- Defensins form channels in bacterial cell membranes
- Lactoferrin chelates iron
- Hydrolytic enzymes

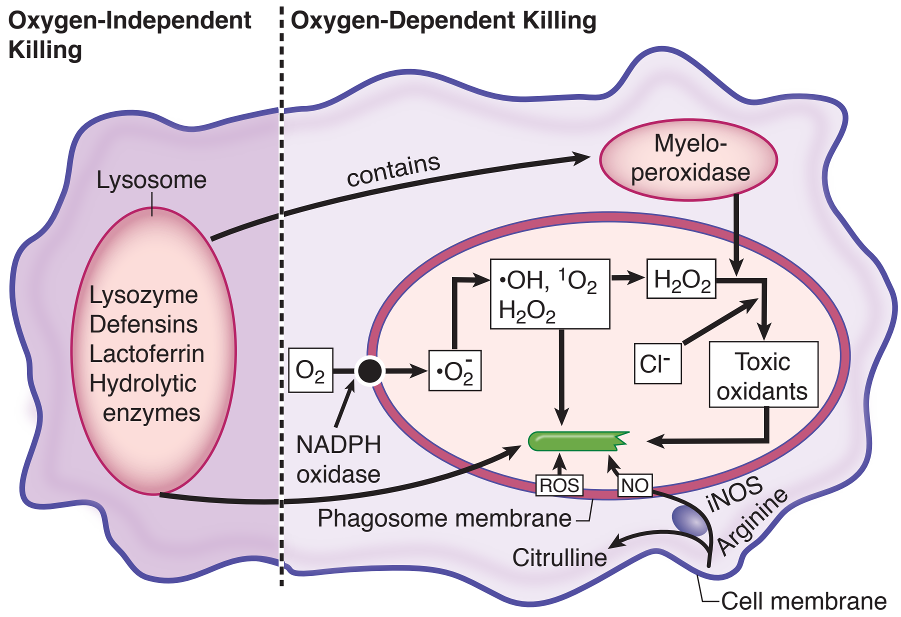


Figure I-4-11. Metabolic Stimulation and Killing Within the Phagocyte

Systemic Inflammation

During the acute inflammatory response, pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α are produced. These cytokines have systemic effects on the tissues, including fever, production of acute phase proteins, and leukocytosis.

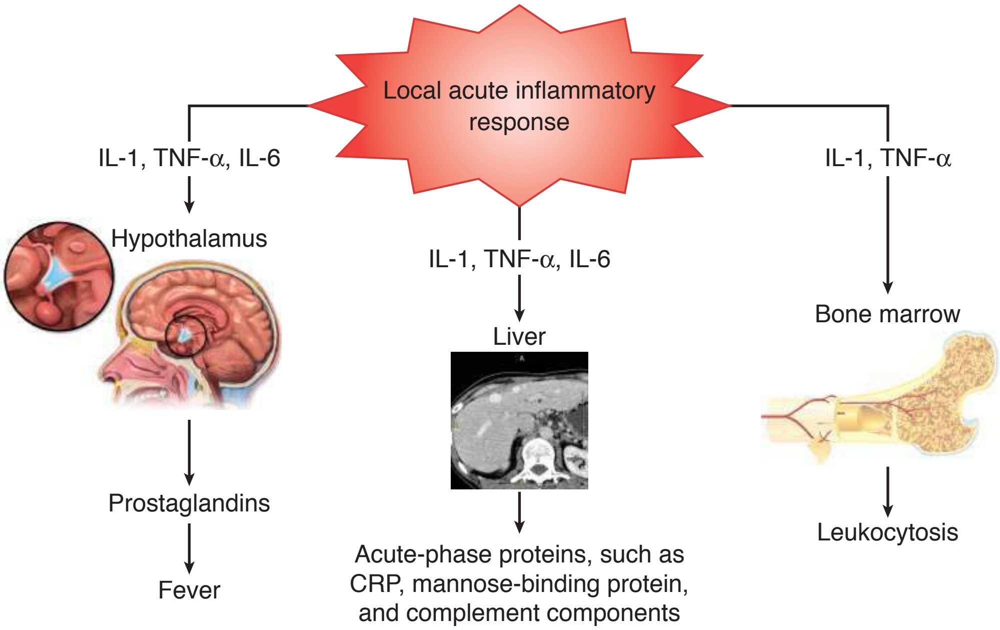


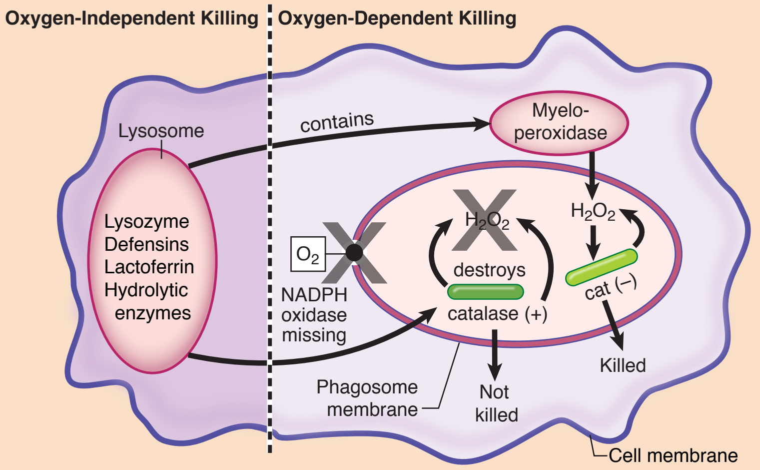
Figure I-4-12. Systemic Inflammatory Response

Clinical Correlate

When defects prevent phagocytes from performing their critical functions as first responders and intracellular destroyers of invading antigens, clinically important pathologic processes ensue. Such defects tend to make the patient susceptible to severe infections with **extracellular bacteria** and **fungi**.

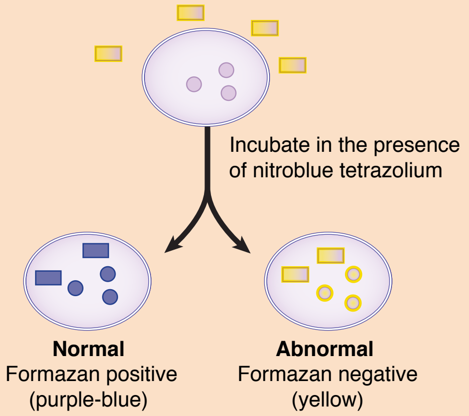
Chronic granulomatous disease (CGD) is an inherited deficiency in the production of one of several subunits of **NADPH oxidase.** This defect eliminates the phagocyte's ability to produce many critical oxygen-dependent intracellular metabolites ($\cdot O_2^-$, $\cdot OH$, $^1O_2$, and H_2O_2). The 2 other intracellular killing mechanisms remain intact (myeloperoxidase + $H_2O_2 \rightarrow$ HOCl and lysosomal contents).

- If the patient is infected with a catalase-negative organism, the H_2O_2 waste product produced by the bacterium can be used as a substrate for myeloperoxidase, and the bacterium is killed.
- If the patient is infected with a catalase-positive organism (e.g., *Staphylococcus, Klebsiella, Serratia, Aspergillus*), the myeloperoxidase system lacks its substrate (because these organisms destroy H_2O_2), and the patient is left with the oxygen-independent lysosomal mechanisms that prove inadequate to control rampant infections. Thus, CGD patients suffer from chronic, recurrent infections with **catalase-positive** organisms.



Intracellular Killing in CGD

Failures of phagocytic cells to generate oxygen radicals are easily detected by the nitroblue tetrazolium (NBT) reduction test or neutrophil oxidative index (NOI; a flow cytometric assay). The dihydrorhodamine test—a similar test using flow cytometry—may also be used.



Nitroblue Tetrazolium Reduction

Table I-4-6. Cytokines Involved in Innate Immunity*

Cytokine(s)		Cell Secreted by	Target Cells/Tissues	Activity
Pro-inflammatory cytokines	IL-1	Macrophages	Hypothalamus	Fever
			Endothelial cells	Increases expression of ICAMs
			Liver	Stimulates production of acute phase proteins
	IL-6	Macrophages	Liver	Synthesis of acute phase proteins
	TNF-α	Macrophages	Hypothalamus	Fever
			Endothelial cells	Increases expression of ICAMs
			Liver	Stimulates production of acute phase proteins
			Neutrophils	Activation
			Tumor cells	Apoptosis
			Fat, muscle	Cachexia
Chemokine	IL-8	Macrophages	Leukocytes	Induces adherence to endothelium, chemotaxis, extravasation
IL-12		Macrophages, dendritic cells	NK cells	IFN-γ production
IL-15		Macrophages	NK cells	Proliferation
IL-18		Macrophages		IFN-γ synthesis
Regulatory	IL-10	Macrophages, dendritic cells	Macrophages, dendritic cells	Inhibition of IL-12 production, decreased expression of co-stimulatory molecules, decreased class II MHC expression
Type I IFNs	IFN-α	Dendritic cells, macrophages	All cells	Transient inhibition of protein synthesis, increased class I MHC expression
			NK cells	Activation
	IFN-β	Fibroblasts	All cells	Transient inhibition of protein synthesis, increased class I MHC expression
			NK cells	Activation
TGF-β		Macrophages, lymphocytes, etc.		Anti-inflammatory

*Only the innate functions of these cytokines are described here. Many of these cytokines have important functions in the regulation of the adaptive immune response; those will be discussed in subsequent chapters.

Innate Response to Viruses

The innate response to viruses is unique in that it is geared toward eliminating these intracellular pathogens. The 2 major mechanisms for dealing with viral infections are IFN-α/β and NK cells.

Interferons

Interferons (IFNs) are a family of eukaryotic cell proteins classified according to the cell of origin. IFN-α and IFN-β are produced by a variety of virus-infected cells. They do the following:

- Act on target cells to inhibit viral replication, not the virus
- Are not virus-specific

Interferon inhibits viral protein synthesis:

- Activation of an RNA endonuclease, which digests viral RNA
- Phosphorylation of protein kinase, which inactivates eIF2, inhibiting viral protein synthesis

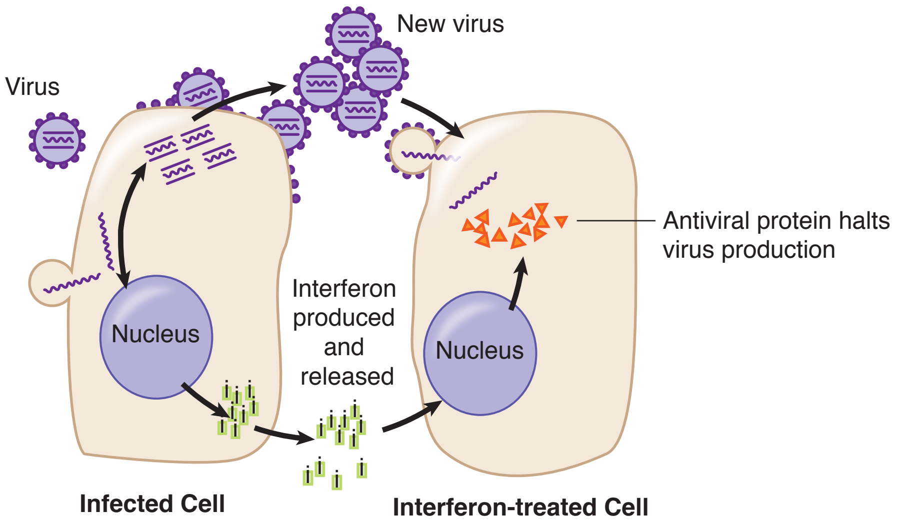


Figure I-4-13. Interferon Production

Clinical Correlate

Therapeutic Use of Interferons

Since the first description of interferons (IFN) almost 50 years ago, a multitude of dramatic immunomodulatory roles have been discovered for this group of proteins. As a group, IFNs induce increases in the expression of class I and II MHC molecules and augment NK cell activity. They increase the efficiency of presentation of antigens to both cytotoxic and helper cell populations. Cloning of the genes that encode IFNs α, β, and γ has made it possible to produce sufficient amounts to make their use clinically practical.

- **Interferon-α** has well-known antiviral activity and has been used in the treatment of hepatitis B and C infections. Within cancer therapy, IFN-α has shown promise in treatment of hairy B-cell leukemia, chronic myelogenous leukemia, and Kaposi sarcoma.
- **Interferon-β** was the first drug shown to have a positive effect on young adults with multiple sclerosis. Patients treated with IFN-β enjoy longer periods of remission and reduced severity of relapses.
- **Interferon-γ** is being used in the treatment of chronic granulomatous disease (CGD). This molecule is a potent inducer of macrophage activation and a promoter of inflammatory responses. Its application appears to significantly reverse the CGD patient's inability to generate toxic oxygen metabolites inside phagocytic cells.

The side effects of IFN therapy are fortunately mild and can be managed with acetaminophen. They include headache, fever, chills, and fatigue, and they diminish with continued treatment.

NK Cells

NK cells are members of the innate branch of the immune response. They exhibit the capacity to kill virally infected cells and tumor cells. They kill via the same mechanisms of inducing apoptosis observed with CTLs (granzymes, perforin). NK activity is increased in the presence of interferons (IFNs) α and β, and IL-12.

NK cells share a common early progenitor with T cells, but they do not develop in the thymus. They do not express antigen-specific receptors or CD3. The markers used clinically to enumerate NK cells include CD16 (FcRγ) and CD56 (CAM). Their recognition of targets is not MHC-restricted, and their activity does not generate immunologic memory.

NK cells employ 2 categories of receptor: **killer activating receptor (KAR)** and **killer inhibitory receptor (KIR)**. If only KARs are engaged, the target cells will be killed. If both the KIRs and the KARs are ligated, the target cell lives. Therefore the inhibitory signals trump the activation signals.

NKG2D is the major KAR expressed by NK cells. There are many ligands for KARs; the MIC glycoproteins are one type. MIC proteins are stress proteins that are expressed only when cells are infected or undergoing transformation. Upon the binding of KAR to a MIC protein, NK cells become cytotoxic, resulting in death of the target cell.

The KIRs activate protein tyrosine phosphatases which inhibit intracellular signaling and activation by removing tyrosine residues from various signaling molecules. The KIRs on the NK cell bind to a specialized type of MHC class I antigens called HLA-E. HLA-E has a ubiquitous tissue distribution, as do the other class I HLA molecules. The HLA-E molecules bind to peptides derived from the leader sequence of HLA-A, -B and –C. HLA-E requires a bound peptide for proper expression within a cell. Therefore, the amount of HLA-E expression on a cell is indicative of the overall well-being of the cell. During viral infections or in transformed cells, the amount of class I HLA expression may be decreased, which would prevent the leader sequences from binding to HLA-E. This would decrease the expression of HLA-E, and make cells susceptible to NK mediated killing.

Interestingly, when NK cells are activated through the FcR (CD16), only one signal is required because the antibody signals that there is an active infection. This occurs through a mechanism called antibody-dependent cell-mediated cytotoxicity (ADCC) (*see* chapter 8).

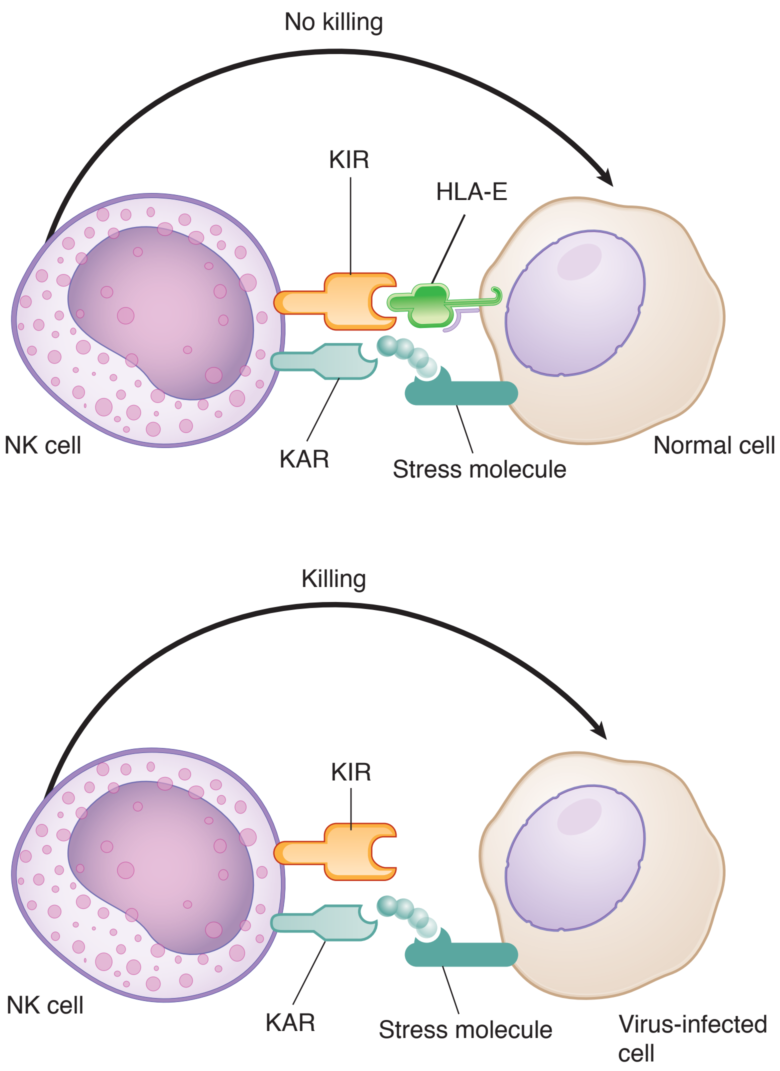


Figure I-4-14. Activation of NK Cells

Secondary Lymphoid Tissue: Innate Immune Response Meets Adaptive

5

Learning Objectives

- ❏ Demonstrate understanding of inflammatory response
- ❏ Solve problems concerning structure of and migration to secondary lymphoid tissue

MIGRATION TO THE SECONDARY LYMPHOID TISSUE

Within a few hours of the initiation of the acute inflammatory response, the professional APCs that have phagocytosed and processed the invading antigen begin to leave the area via lymphatic vessels. Dendritic cells are probably the most efficient of these cells and retract their membranous processes to round up and begin the journey to the closest lymph node (Figure I-5-1).

As discussed earlier, dendritic cells and other phagocytes such as macrophages bind to antigens via PRRs, with a limited diversity, such as the TLRs. The activation of the TLRs induces an acute inflammatory response in the tissue, leading to the production of pro-inflammatory cytokines. These cytokines cause a change in the phenotype of the phagocyte which eventually alters their migration pattern and enhances their function.

Activated dendritic cells will begin to express a chemokine receptor called CCR7. CCR7 is activated by chemokines that are produced by the endothelium.

- Chemokines bind to CCR7 on DCs, allowing them to exit the tissue.
- Upon activation, DCs switch focus from antigen-capture to antigen-presentation.
- Activated DCs concentrate in draining lymph nodes and become trapped in the paracortex.
- Naive T cells expressing CCR7 bind to chemokines on HEVs and migrate to the paracortex.

Considering the vast number of pathogens that enter the body, it would be a nearly impossible task for the lymphoid cells to travel to all body sites to protect the host. Thus, the antigens are taken to the secondary lymphoid tissues where the lymphocytes constantly recirculate in order to come into contact with their specific antigen.

If the initial tissue damage is sufficient, these cells can also be flushed into blood vessels, ultimately becoming trapped in the vascular sinusoids of the spleen. Regardless, the secondary lymphoid organs (lymph nodes and spleen) are the sites where naive, mature lymphocytes will first be exposed to their specific antigens.

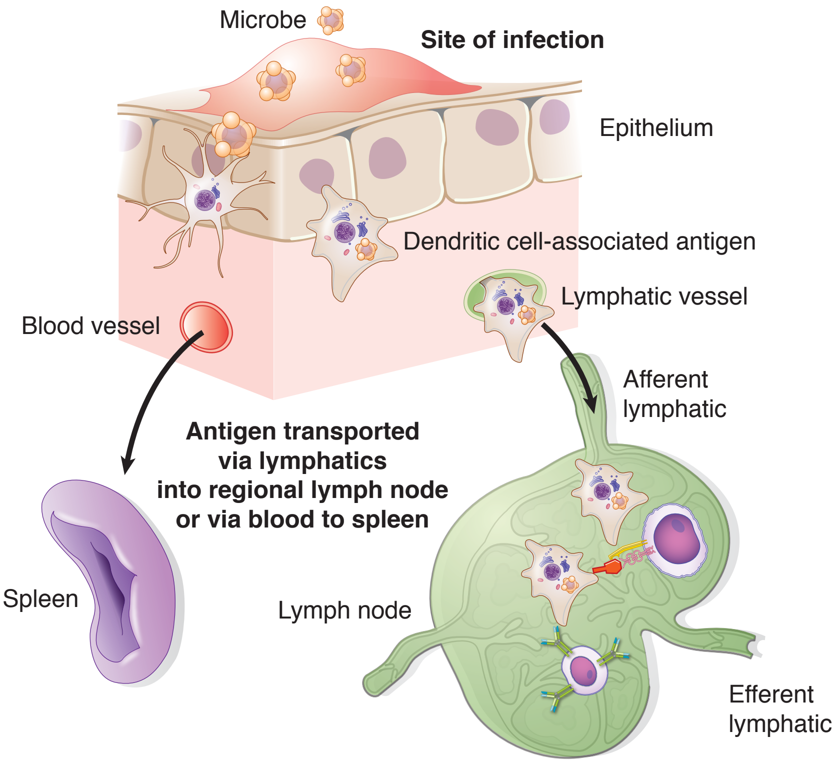


Figure I-5-1. Transportation of Antigen to the Secondary Lymphoid Organs

STRUCTURE OF THE SECONDARY LYMPHOID TISSUE

Lymph nodes are small nodular aggregates of secondary lymphoid tissue found along the lymphatic channels of the body. They are designed to initiate immune responses to tissue-borne antigens.

- Each lymph node is surrounded by a fibrous capsule that is punctured by afferent lymphatics, which bring lymph into the subcapsular sinus.
- The fluid percolates through an outer cortex area that contains aggregates of cells called follicles.
- The lymph then passes into the inner medulla and the medullary sinus before leaving the node through the hilum in an efferent lymphatic vessel.
- Ultimately, lymph from throughout the body is collected into the thoracic duct, which empties into the vena cava and returns it to the blood.

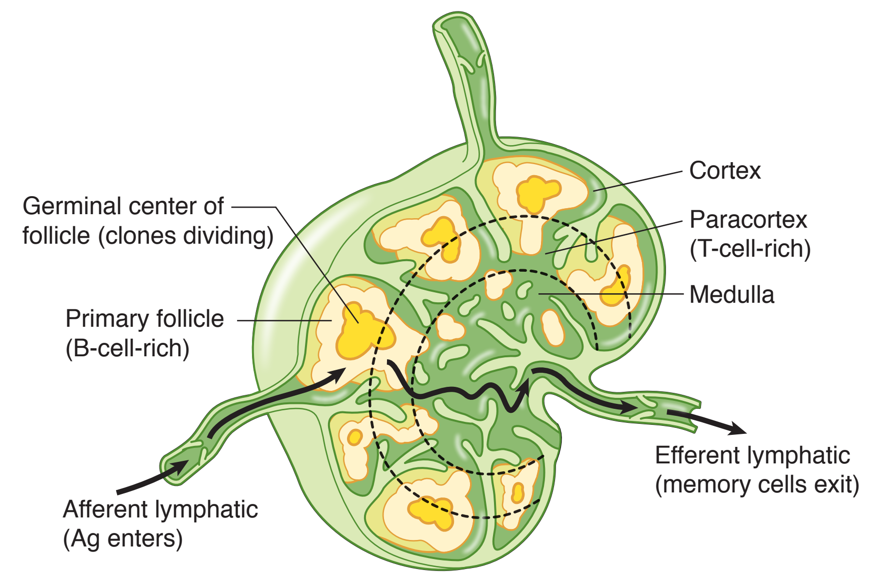


Figure I-5-2. Compartmentalization of a Lymph Node

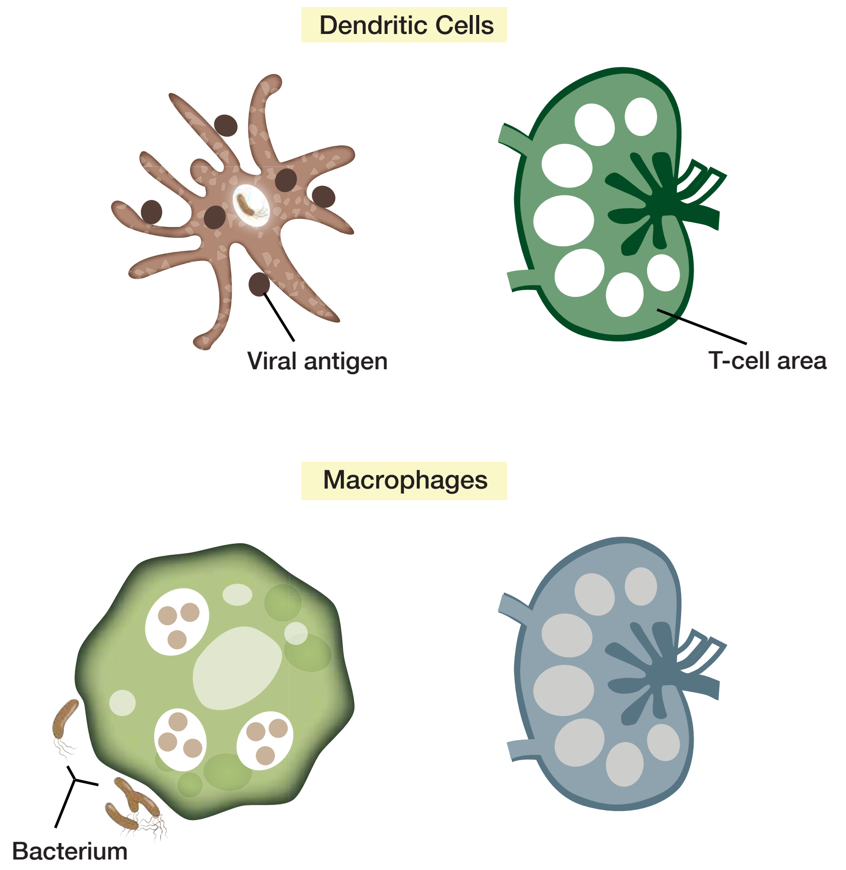


Figure I-5-3. Location of Macrophages and DCs in Secondary Lymphoid Tissue

The spleen is the secondary lymphoid organ that initiates immune responses to blood-borne antigens. A single splenic artery enters the capsule at the hilum and branches into arterioles, which become surrounded by cuffs of lymphocytes known as the **periarteriolar lymphoid sheaths (PALS)**. Lymphoid follicles surrounded by a rim of lymphocytes and macrophages are attached nearby. This constitutes the white pulp. The arterioles ultimately end in vascular sinusoids, which make up the red pulp. From here, venules collect blood into the splenic vein, which empties into the portal circulation.

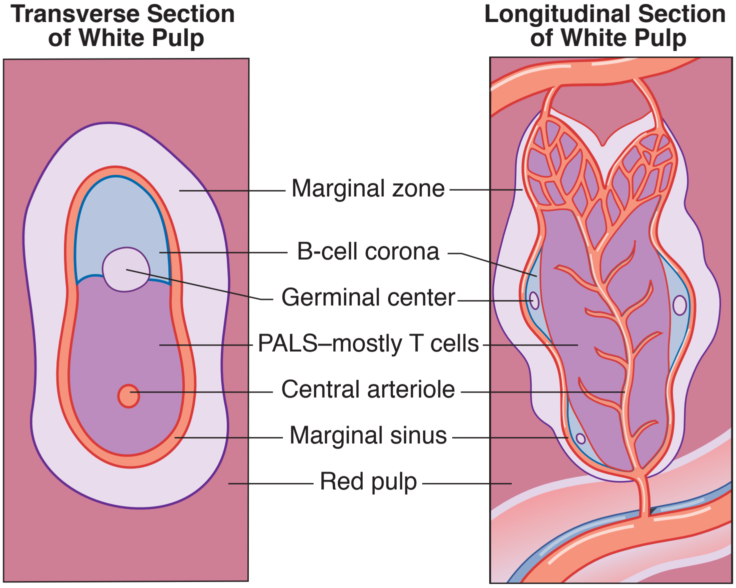


Figure I-5-4. Structure of the Spleen

ANTIGEN PROCESSING AND PRESENTATION

Exogenous Pathway of MHC Loading

Although some small, easily digestible antigens are almost totally degraded and exocytosed by phagocytes, the critical first step in the elicitation of the adaptive immune response to a primary antigenic challenge is the processing of antigen for the presentation to naive T lymphocytes.

Professional antigen-presenting cells (APCs) include dendritic cells, macrophages, and B cells; their job is to load partially degraded peptides into the groove of the MHC class II molecules.

The APCs have slightly different functions to help elicit unique immune responses required to eliminate various types of pathogens.

- **Dendritic cells** are the most prolific of the APCs, as they do not have to be activated in order to present antigen to T cells. They constitutively express the co-stimulatory molecules needed to activate the T cells.
- **Macrophages** help activate the Th1 response by digesting microbes and presenting them to the T cells to elicit a cell-mediated immune response (*see* chapter 8).

- **B cells** present specific protein antigens to T cells to help elicit a humoral immune response or a Th2 response (*see* chapter 7). B cells are unique, as they are the only APCs that specifically recognize antigen via the B cell receptors (of surface bound antibody).

Table I-5-1. APC Expression of Co-stimulatory Molecules, Class II MHC, and Function

APC	Expression of Co-stimulatory Molecules	Expression of HLA Class II	Major Function
Dendritic cells	Constitutive: B7 (B7.2)*, CD40 Inducible: IFN-γ, TLRs	Constitutive but upregulated by IFN-γ	Activation of naïve Th cells
Macrophages	Constitutive: B7 (B7.2), CD40 Inducible: IFN-γ, TLRs	Negative or low level expression but induced by IFN-γ	Initiation and effector phase of the T_h1 response for cell mediated immunity
B cells	Constitutive: CD40 Inducible: T cells, B7	Constitutive but upregulated by IL-4	Initiation of the T_h2 response for humoral immunity

*B7.2 is also called CD86.

When MHC class II molecules are produced in the endoplasmic reticulum of an APC, a protein called the invariant chain (Ii) is synthesized at the same time. The Ii has a class II invariant chain peptide (CLIP) that binds with high affinity to the peptide binding cleft of newly synthesized MHC class II molecule. The Ii with its associated CLIP blocks the peptide-binding groove so no normal cellular peptides can accidentally be attached. The CLIP + Ii is transported in a vesicle to the location of endocytic vesicles containing the ingested, internalized peptides.

A molecule called HLA-DM is found within the late endosome (lysosomal compartment). It is the job of HLA-DM to exchange the CLIP for a phagocytosed peptide that will bind to the MHC class II molecule with even higher affinity than the CLIP. Once exchanged for the CLIP, the peptide is loaded on the MHC class II molecule and the complex is transported to the cell surface, where it will be accessible for interaction with any T lymphocyte with a complementary TCR. If, however, the class II molecule does not find a peptide that it can bind with even higher affinity than the CLIP, the empty class II molecule is unstable and degraded, and will thus never make it to the cell surface.

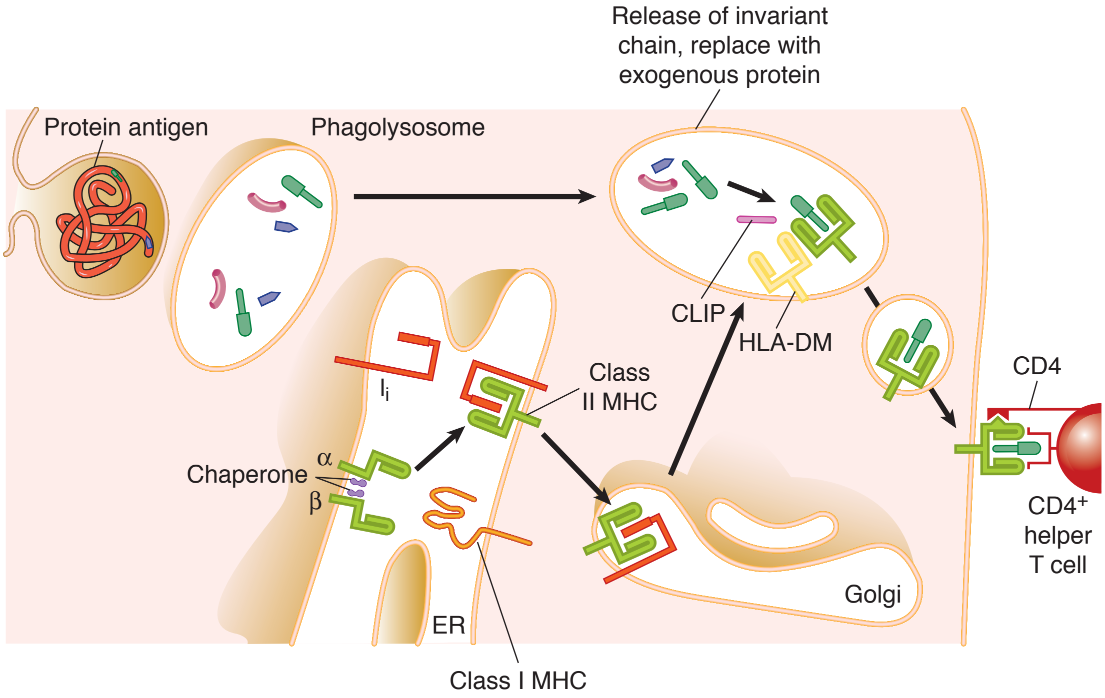


Figure I-5-5. Exogenous Pathway of Antigen Presentation

Endogenous Pathway of MHC Loading

The endogenous pathway of antigen-processing handles threats to the host which are intracellular. These might include viruses, altered/mutated genes (from tumors), or even peptides from phagocytosed pathogens that may escape or be transported out of phagosomes into the host cell cytoplasm. Intracellular proteins are routinely targeted by ubiquitin and degraded in proteasomes.

The peptides from these proteins are transported through a peptide transporter known as the **TAP complex (transporter of antigen processing)**, and into the endoplasmic reticulum, where they have the opportunity to bind to freshly synthesized MHC class I molecules.

- The TAP complex includes the TAP proteins that form the tunnel through which the proteins travel and a bridging protein called tapasin.
- Tapasin bridges the TAP transporter to the MHC class I molecule so that as these peptides enter the endoplasmic reticulum, they are easily bound by the newly synthesized and empty class I molecules.
- The peptide-MHC class I complexes are then transported to the cell membrane where they may be presented to CD8+ T lymphocytes (*see* chapter 8).

Just as with the MHC class II molecules, MHC class I is unstable without the addition of peptide and will not be expressed at the cell surface without the addition of peptide.

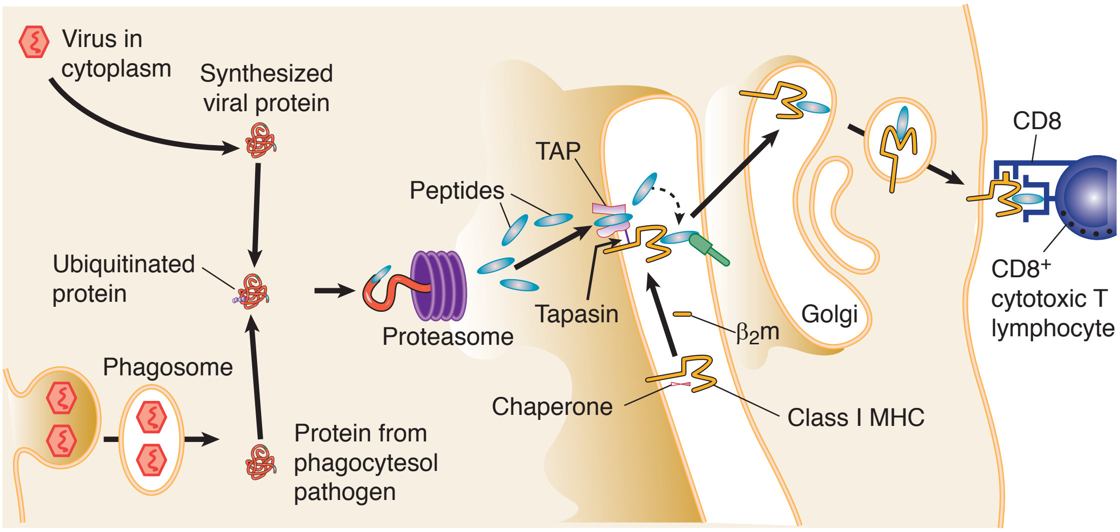


Figure I-5-6. Endogenous Pathway of Antigen Presentation

Clinical Correlate

Proteasome Inhibitors in the Treatment of Cancer

By their very nature, oncogenic cells are overly proliferative, requiring a higher rate of protein synthesis than their normal cell counterparts. A majority of cellular proteins are degraded via the ubiquitin proteasome pathway, including many proteins that play a role in maintaining cellular homeostasis. These include proteins that regulate the cell cycle, apoptosis, etc.

- Proteasome inhibitors induce apoptosis in tumor cells by interfering with the degradation of these regulatory proteins. For example, proteins that regulate the cell cycle such as p53 may be inactivated in transformed cells. This leads to a dysregulation of cell cycle control and a progression of the tumorigenesis.
- Proteasome inhibitors will produce an accumulation of p53 as well as other regulatory proteins, and therefore eventual cell death via apoptosis.

Table I-5-2. FDA-Approved Proteasome Inhibitors in Clinical Use

Drug Name	Use
Bortezomib	Currently approved to treat multiple myeloma and mantle cell lymphoma (clinical trials for leukemia)
Carfilzomib	Currently approved to treat multiple myeloma (clinical trials for leukemia and lymphomas)

Cross Presentation (Cross Priming)

In addition to presenting antigens on MHC class II molecules to CD4+ T cells, dendritic cells also have a role in presenting antigens to CD8+ T cells in a process called **cross presentation**. As professional phagocytes, dendritic cells are able to ingest a virally MHC class I infected cell in toto and present viral antigens within a molecule to CD8+ T cells. Therefore, DCs may activate or prime both CD4+ T cells and CD8+ T cells specific for the same pathogen. This activation may occur in close proximity, which is important for the activation of naïve CD8+ T cells into activated CTLs and memory cells. This occurs via the activation of CD4+ T cells and the production of cytokines such as IL-2 (*see* chapter 8).

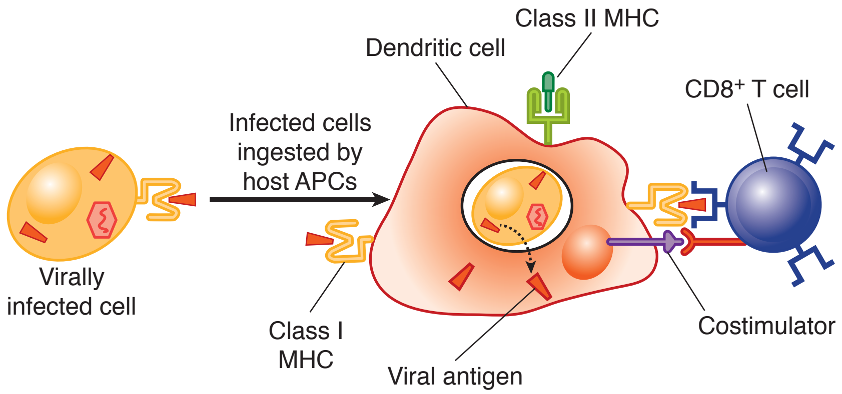


Figure I-5-7. Cross-Priming

Secondary Lymphoid Tissue: B and T Lymphocyte Activation

6

Learning Objectives

- ❏ Answer questions about antigen processing and presentation
- ❏ Explain activation of T and B lymphocytes

ACTIVATION OF T LYMPHOCYTES

Once antigen is processed and presented to a T cell, the adaptive immune response is initiated. These interactions occur within the secondary lymphoid tissue. The purpose of these interactions is to generate effector cells, which will ultimately result in the elimination of the infection.

In order to generate specific effector cells, the activation of T cells via the TCR must go through several checkpoints to ensure antigen specificity and eventual T-cell activation.

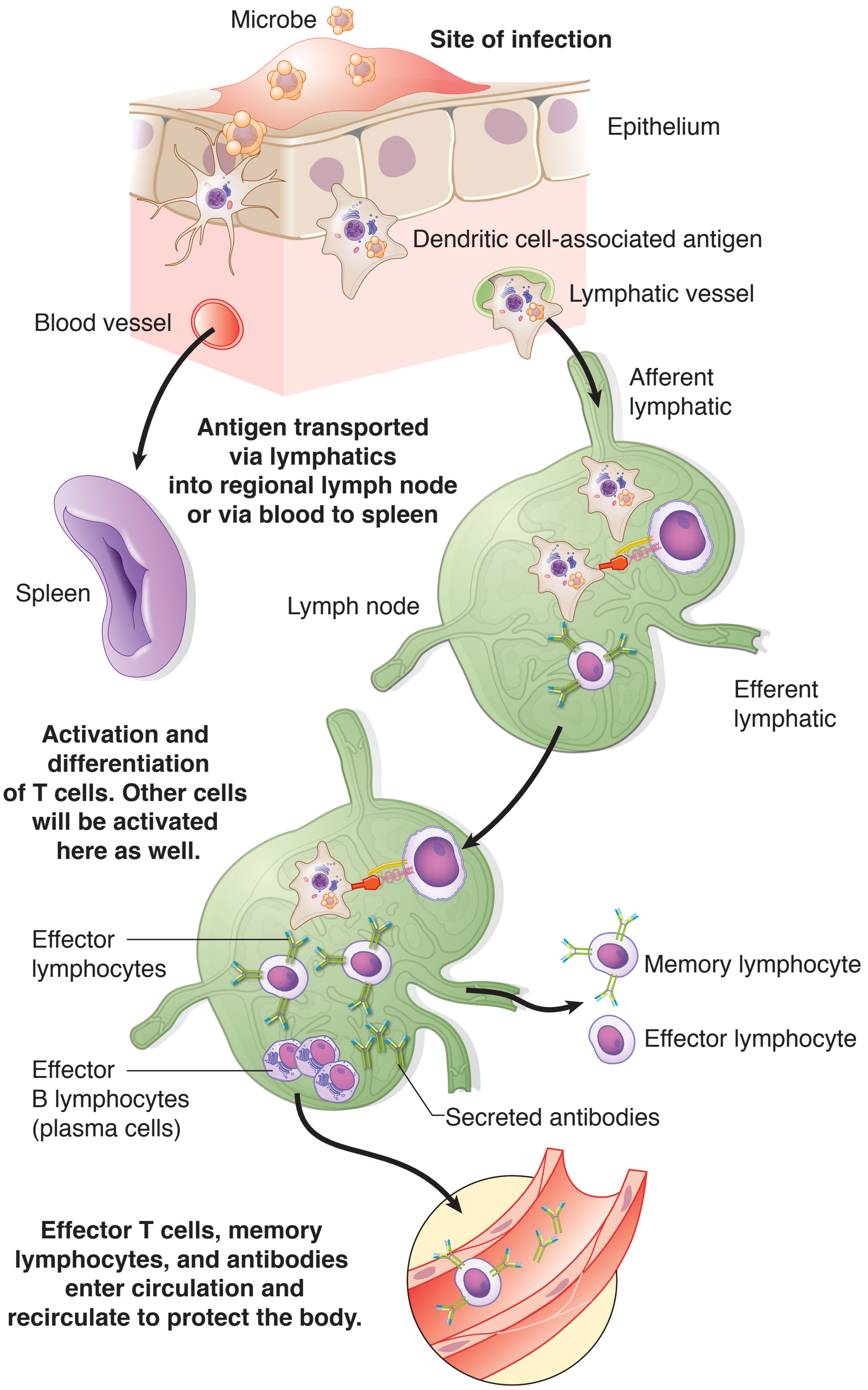


Figure I-6-1. Production of Effector Cells in the Secondary Lymphoid Organs

The binding of the TCR of the mature, naive T cell to the MHC peptide complex of the APC provides the first signal to the T cell to begin its activation. This provides the antigenic specificity of the response. The interaction is stabilized by the coreceptors CD4 and CD8 which bind to MHC class II and MHC class I molecules, respectively.

Intimately associated with the T cell receptor is the CD3 signal transduction complex. Interaction of cell adhesion molecules on the surface of the APCs and T cells allows for the formation of the immune synapse.

The costimulatory molecules B7-1 (CD80) and B7-2 (CD86) on APCs bind to CD28 on the mature, naïve T cells, providing the second signal necessary for successful activation. Under normal conditions, B7 is expressed at low levels on APCs. In the presence of infection or inflammation, the expression will increase, enhancing activation of the mature, naïve T cells. Later in the immune response, B7 will preferentially bind to CTLA-4 or PD-1, effectively turning off the T-cell response.

Cytokines secreted by APCs and the activating T cells themselves induce the proliferation (**clonal expansion**) and differentiation of the T cells into **effector cells** and **memory cells**.

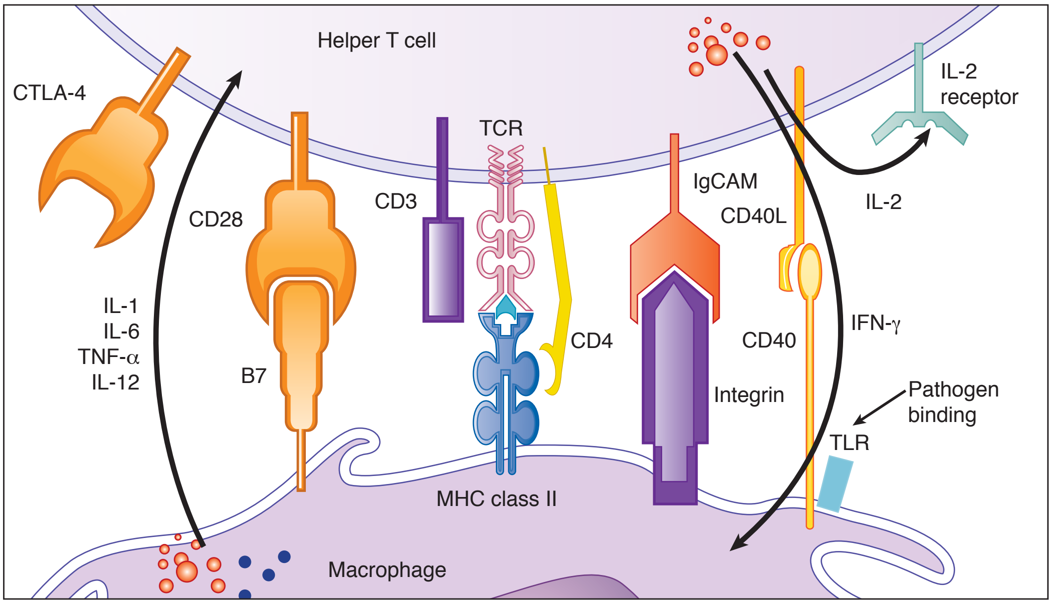


Figure I-6-2. Helper T Cell and Macrophage Adhesion

Several surface molecules are involved in the activation of mature, naive T lymphocytes:

- **First (primary) signal**: recognition of the MHC:peptide complex by the T cell receptor and coreceptors (CD4 and CD8)
- **Second (costimulatory) signal**: recognition of B7 by CD28

Clinical Correlate

CTLA-4 is an important immunoregulatory molecule in the immune system. Expressed on both activated Th and Tregs, CTLA-4 is responsible for downregulating the immune response by competitive binding to B7-1 and B7-2 on APCs.

The manipulation of CTLA-4 has important clinical implications first elucidated by the creation of CTLA-4 knockout mice that resulted in a fatal lymphoproliferative disorder. These deletions may also result in several autoimmune disorders.

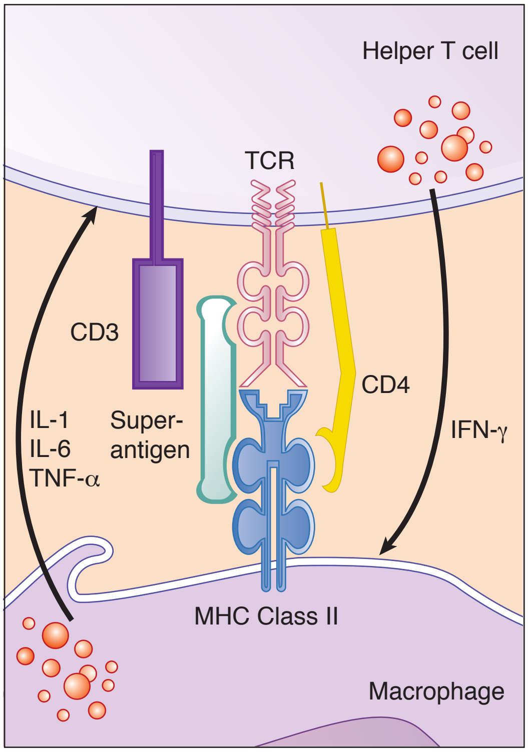
Type of Regulation of CTLA-4	Drug Name	Clinical Use
Agonists	Abatacept	Rheumatoid arthritis
	Belatacept	Renal transplants
Antagonists	Ipilimumab	Melanoma and in clinical trials for several other types of cancer

The activated CD4+ (helper) T lymphocytes will begin to produce and secrete cytokines and increase surface expression of cytokine receptors. The first cytokine produced is IL-2, an autocrine signal, which induces T-cell proliferation by binding to a high affinity IL-2 receptor found on the same cells. Unlike helper T lymphocytes, activated CD8+ T lymphocytes secrete low levels of IL-2 and are dependent on the helper T lymphocytes for their proliferation and differentiation.

Clinical Correlate

Superantigens are viral and bacterial proteins that cross-link the variable β domain of a T-cell receptor to an α chain of a class II MHC molecule outside the normal peptide-binding groove. This cross-linkage provides an activating signal that induces T-cell activation and proliferation, in the absence of antigen-specific recognition of peptides in the MHC class II groove.

Because superantigens bind outside of the antigen-binding cleft, they activate any clones of T cells expressing a particular variable β sequence and thus cause polyclonal activation of T cells, resulting in the over-production of IFN-γ. This, in turn, activates macrophages, resulting in overexpression of proinflammatory cytokines (IL-1, IL-6 and TNF-α). Excess amounts of these cytokines induce systemic toxicity. Molecules produced during infectious processes and known to act as superantigens include staphylococcal enterotoxins, toxic-shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins.



Superantigen Activation

Note that there is no complementarity between the TCR and MHC/peptide complex.

Development of the Th1, Th2, and Th17 Subsets

Helper T lymphocytes serve as the orchestrators of virtually all the possible **effector mechanisms** that will arise to destroy the pathogen. The effector mechanisms that are controlled by Th cells include antibody synthesis, macrophage activation and CTL killing. The "decision" as to which of these mechanisms should be engaged is based on the characteristics of the invading pathogen and is controlled by the differentiation of specialized classes of helper T cells. All CD4+ T cells require recognition of their specific antigen complexed to MHC class II by their TCR (first signal) and costimulation through the binding of B7 on the professional APC by CD28 (costimulatory signal).

There are 3 major classes of helper T (Th) cell that arise from the same precursor, the naive Th lymphocyte (or Th0 cell):

- Th1
- Th2
- Th17

The pattern of differentiation is determined by the antigen or type of pathogen causing the infection, the cytokines produced in response to the antigen, and the transcription factors stimulated by the cytokines.

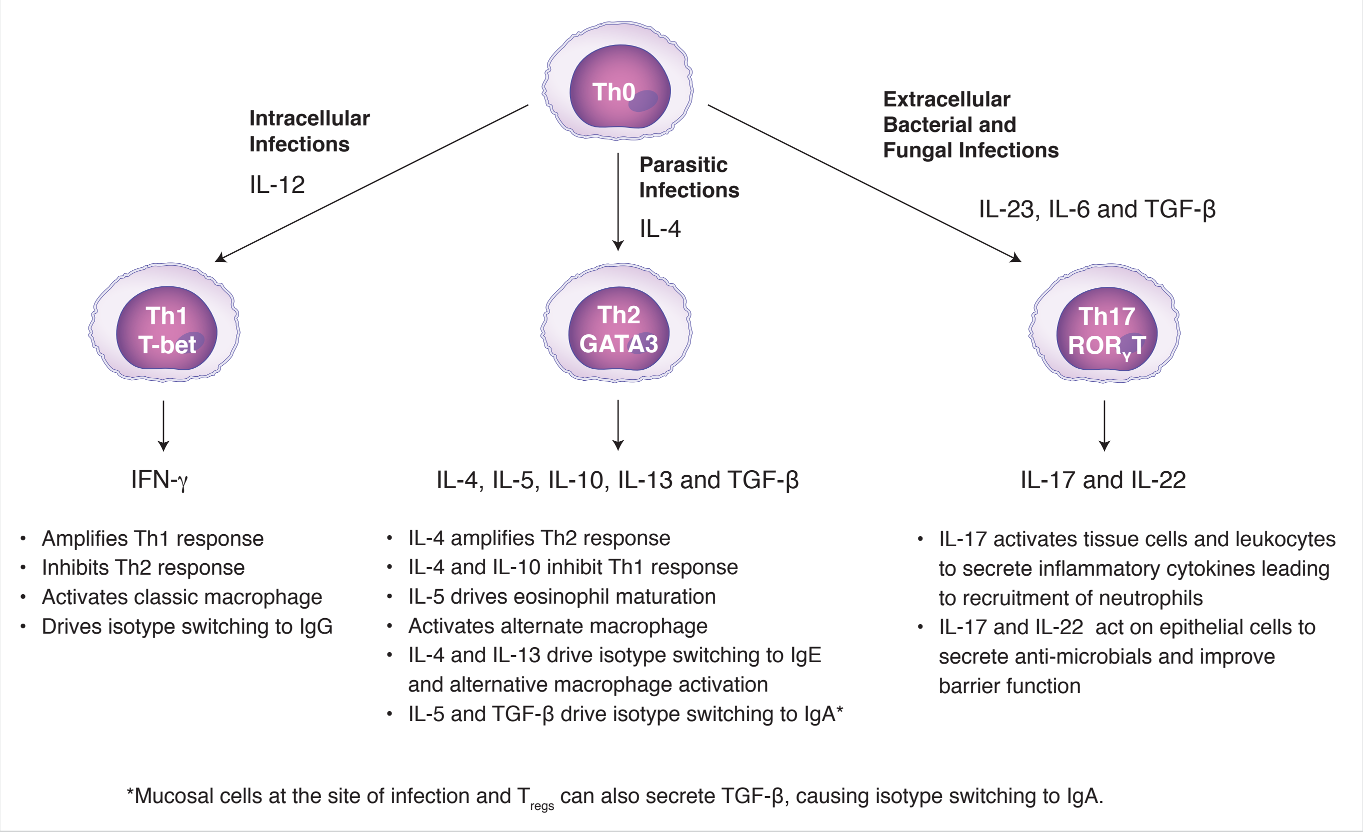


Figure I-6-3. Subsets of Helper T Cells

Differentiation of a Th0 cell into a Th1 cell is stimulated by intracellular pathogens (e.g., viruses and intracellular bacteria). These pathogens induce a strong innate immune response with the resultant production of **IL-12** by macrophages and **IFN-γ** by NK cells increasing the expression of the transcription factor T-bet.

In turn, Th1 cells secrete high levels of the inflammatory cytokine IFN-γ which does the following:

- Amplifies the Th1 response
- Inhibits the Th2 response
- Activates classical macrophage
- Enhances isotype switching to IgG

Differentiation of a Th0 cell into a Th2 cell seems to be encouraged in response to large extracellular parasites such as helminths or allergens. Due to the inability to phagocytose these pathogens, there is not significant macrophage or NK-cell stimulation. In this way, naive Th0 cells seem to produce IL-4 constitutively, and in the absence of IL-12 stimulation, these cells will upregulate their production of IL-4 to encourage differentiation into Th2 cells by induction of the transcription factor GATA-3. Additional IL-4 is produced by the activation of mast cells and eosinophils by the helminths or allergens further driving differentiation into Th2 cells.

Several cytokines are produced by Th2 cells, including IL-4, IL-5, IL-10, IL-13 and TGF-β.

- IL-4 causes B lymphocytes to isotype switch predominantly to IgE, which will bind to mast cells, eosinophils and basophils.
- In collaboration with IL-13, IL-4 enhances alternative macrophage activation for tissue repair and increased intestinal mucus secretion and peristalsis.
- The combination of IL-4, IL-10, and IL-13 together promotes a Th2 response while inhibiting the Th1 response.
- IL-5 drives maturation and activation of eosinophils.

Differentiation of a Th0 cell into a Th17 cell occurs in the presence of extracellular bacterial and fungal infections. Local cells react to the infection by secreting IL-1, IL-6, IL-23, and TGF-β, inducing the expression of the transcription factor RORγ-T in the Th17 cells. The activated Th17 cells will in turn secrete the cytokines IL-17 and IL-22.

- IL-17 induces local cells to increase chemokine production recruiting neutrophils.
- IL-22 stabilizes interactions between cells in the endothelium decreasing permeability.
- IL-17 and IL-22 induce secretion of anti-microbials by the endothelium.

Another population of T cells that arises from the Th0 is the T regulatory cell (T_{Reg} cell). T_{Reg} cells regulate (inhibit) Th1 cell function.

- Identified by their constitutive expression of CD25 on the surface and by the expression of the transcription factor FoxP3
- Secrete inflammation inhibiting cytokines such as IL-10 and TGF-β
- Have been shown to be critical for the prevention of autoimmunity

Development of Cytotoxic T Lymphocytes

Like CD4+ T cells, CD8+ T cells require both a primary and a costimulatory signal to become activated. The main difference between them is that CD8+ T cells recognize their specific antigen presented by MHC class I molecules and rely upon the cytokines produced by T helper cells to proliferate and ultimately differentiate into cytotoxic T lymphocytes (CTLs).

Clinical Correlate

Tuberculoid vs. Lepromatous Leprosy

The progression of disease with *Mycobacterium leprae* in humans is a well-documented example of the crucial balance between Th1 and Th2 subsets. Leprosy is not a single clinical entity, but rather a spectrum of diseases, with tuberculoid and lepromatous forms at the far poles.

- In **tuberculoid leprosy**, the patient has a strong Th1 response, which eradicates the intracellular pathogens by granuloma formation. There is some damage to skin and peripheral nerves, but the disease progresses slowly, if at all, and the patient survives.
- In **lepromatous leprosy**, the Th2 response is turned on, and because of reciprocal inhibition, the cell-mediated response is depressed. Patients develop antibodies to the pathogen that are not protective, and the mycobacteria multiply inside macrophages, sometimes reaching levels of 1010 per gram of tissue. Hypergammaglobulinemia may occur, and these cases frequently progress to disseminated and disfiguring infections.

ACTIVATION OF B LYMPHOCYTES

As mature naive B lymphocytes leave the bone marrow following successful rearrangement of their membrane immunoglobulin receptor genes, they recirculate throughout the body, attracted to **follicular areas** of the lymph nodes and spleen. If antigen entering these secondary lymphoid organs binds to and cross-links the idiotypes of the immunoglobulin, this provides the first signal for the activation of the B lymphocyte.

The antigens that B lymphocytes encounter are divided into 2 categories**: thymus-independent** (TI) antigens and **thymus-dependent** (TD) antigens.

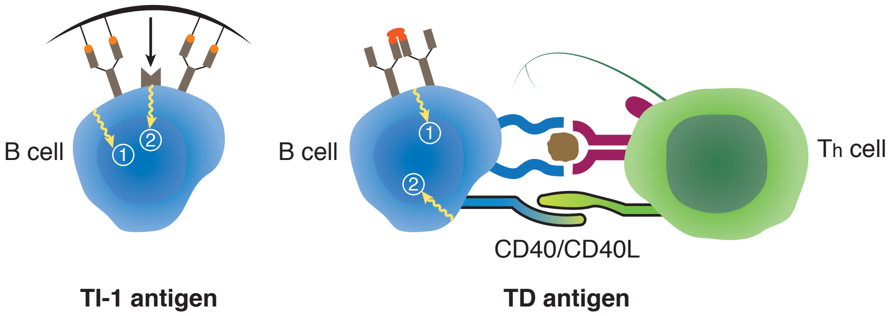


Figure I-6-4. TI versus TD Antigens

TI-Antigen Activated B Lymphocytes

Certain mature, naïve B lymphocytes are capable of being activated by macromolecules such as lipids, polysaccharides, and lipopolysaccharides without having to interact with helper T cells. These antigens are called **thymus-independent (TI)** antigens, and they directly stimulate B cells to proliferate and differentiate into plasma cells.

- The response to TI antigens is generally **weaker** than the response to TD antigens, resulting primarily in the **secretion of IgM antibodies** and the **absence of immunologic memory**.
- TI antigens may also act as B-cell **mitogens**, directly causing mitosis regardless of the cell's antigenic specificity.
- B lymphocytes activated by TI antigens are found in the spleen and mucosa.
- The marginal zone B cells are found in the periphery of the splenic white pulp and the B-1 cells in association with the mucosa and peritoneum.

TD-Antigen Activated B Lymphocytes

Most antigens introduced in the body fall into the category of **thymus-dependent (TD) antigens**. Response to such molecules requires the direct contact of B cells with helper T cells and are influenced by **cytokines** secreted by these cells. After the cross-linking of receptors on the B-cell surface with antigen, the material is endocytosed and processed via the exogenous pathway to generate an MHC class II:peptide complex, which is then inserted into the membrane of the professional APCs.

Simultaneously, expression of B7 is upregulated on the B lymphocytes, making the cells effective presenters of antigen to CD4+ T cells in the area. Once a CD4+ T cell recognizes its specific peptide displayed on MHC class II molecules, the 2 cells form a **conjugate**.

- The CD4+ T cell is activated and differentiates into a helper T cell.
- The helper T cells rearrange their Golgi apparatus toward the junction with the B cell leading to the directional release of cytokines.
- Expression of CD40L on the surface of the helper T cell is upregulated and interacts with CD40 on the B cell to provide the second signal for B-cell activation.
- The B cells respond by proliferating and differentiating into plasma cells and memory B cells.

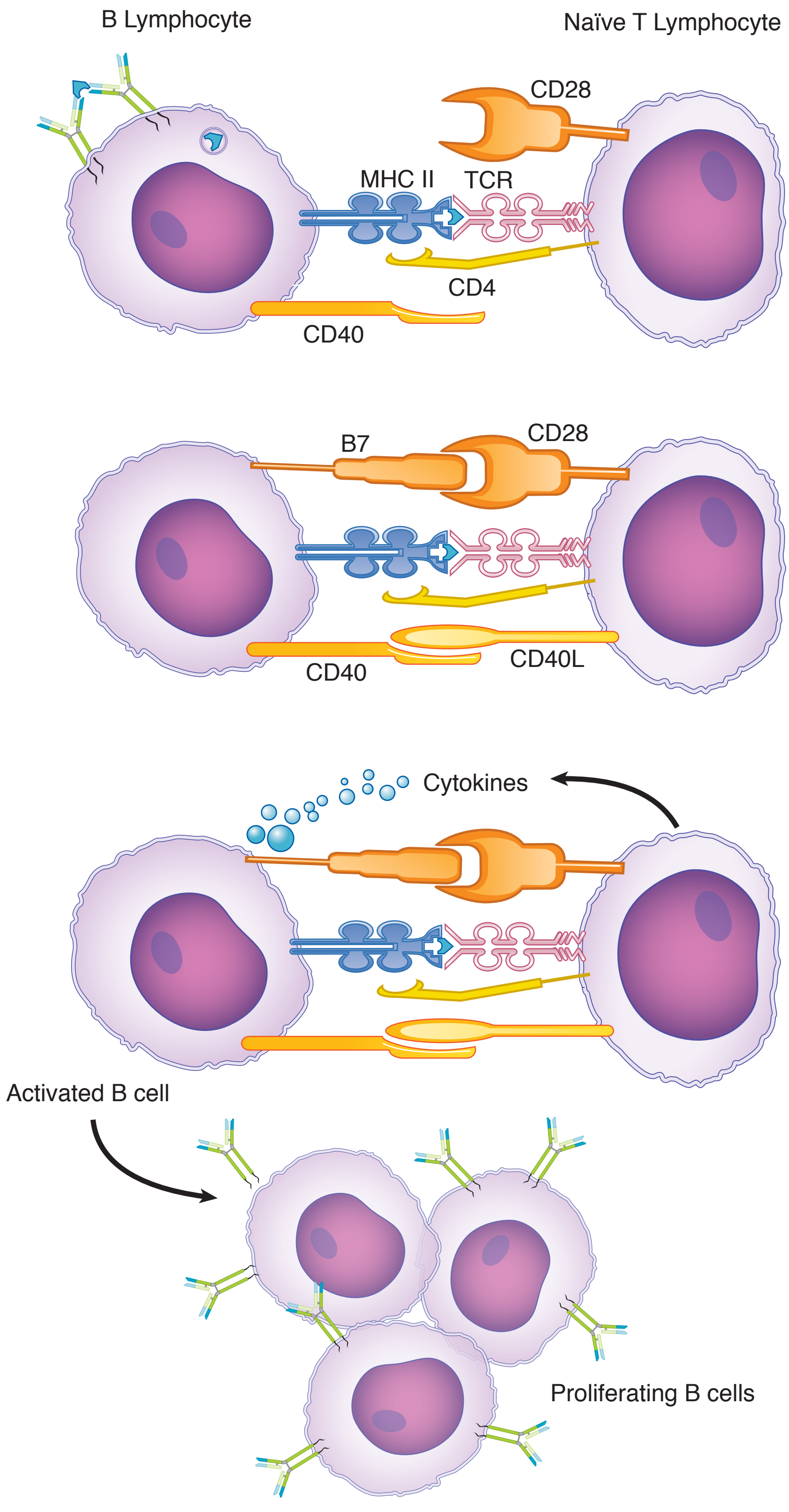


Figure I-6-5. Steps in T-Cell-Dependent B-Cell Activation

B lymphocytes activated by TD antigens are released in 2 subsequent waves.

- The primary wave of activated B lymphocytes is comprised of strictly IgM-secreting plasma cells which leave the secondary lymphoid tissue shortly after being activated.
- The second wave of activated B lymphocytes remains within the follicles of the secondary lymphoid tissue undergoing clonal expansion producing the germinal center. During the expansion, the clones will undergo affinity maturation and isotype switching.

Affinity Maturation

During the activation of B lymphocytes by helper T cells, intense proliferation of the B cells results in the formation of **germinal centers** in the follicles of the secondary lymphoid tissues. These are clones of proliferating, antigen-specific cells. During the intense proliferative response of the B cell, random mutations in the coding of the variable domain region may occur. This is called **somatic hypermutation** and creates single point mutations in the antibody idiotype. If these slightly altered idiotypes have increased affinity for the antigen, then the cell expressing them will be at a selective advantage in competing to bind antigen.

Because binding antigen serves as the first signal for proliferation, over time clones of cells with higher receptor affinity will begin to predominate in the germinal center. This **clonal selection** results in the predominance of clones capable of producing antibodies with increasing affinity for the antigen, a process known as **affinity maturation**.

This means that although isotype switching will necessarily **decrease the avidity** of the preponderance of antibody molecules as the immune response evolves, this will be substituted by an **increase in antibody affinity** over time.

Isotype Switching

Although all of the antibody molecules secreted by a clone of B lymphocytes will have an identical idiotype (*see* chapter 3), the B cell is induced to make new classes (**isotypes**) of immunoglobulin in response to cytokine-directed instruction from the helper T cells.

As the B lymphocyte receives cytokine signals from the helper T cells in the secondary lymphoid organs, it is induced to undergo **isotype switching**, changing the heavy-chain constant domains to classes of antibodies with new and different effector functions. It does this by rearranging the DNA encoding the constant regions of the heavy chain by activating switch regions that cause the intervening DNA to be looped out, excised, and degraded. The idiotype is then joined to a new constant region domain, resulting in an antibody molecule with identical antigenic specificity but a new effector function. This isotype switch is **one-way**; the excised DNA is degraded so a cell that has begun to produce an isotype downstream from IgM coding can never produce IgM again.

This is why IgM is the principal immunoglobulin of the **primary immune response** when antigen is first encountered, and it is replaced in later responses by antibodies of different isotypes. Although IgM antibodies are occasionally produced at low levels during secondary and later immunologic responses, they are always produced by cells encountering that antigen for the first time; namely, mature, naive B cells newly emerging from the bone marrow.

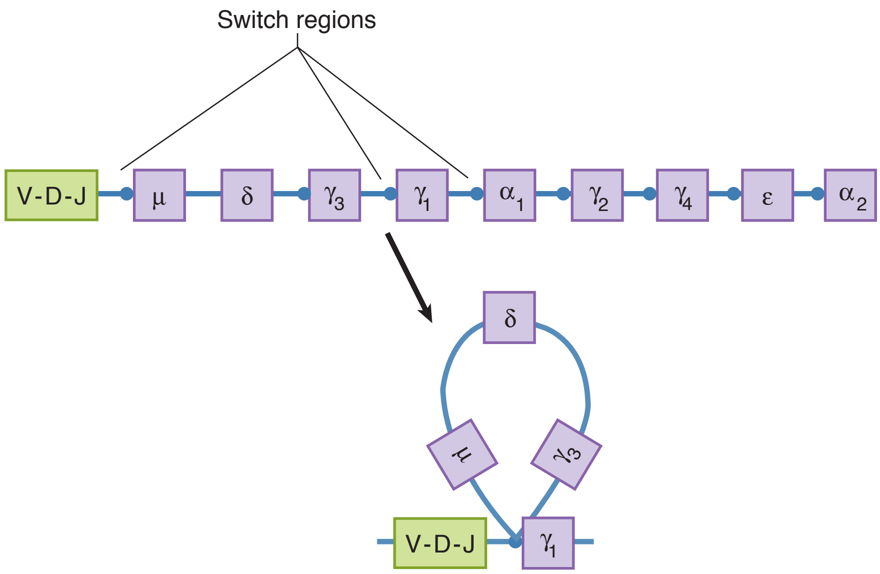


Figure I-6-6. Immunoglobulin Heavy Chain Switching

Humoral Immunity 7

Learning Objectives

- ❏ Explain information related to the primary humoral response
- ❏ Answer questions about antibodies of secondary immune responses

PRIMARY HUMORAL RESPONSE

The first isotype of immunoglobulin that can be produced by a B cell with or without T-cell help is IgM. This is because coding for the constant domains of the heavy chain of IgM (μ chains) are the first sequences downstream from the coding for the idiotype of the molecule.

The IgM molecule on the surface of the B cell is a monomer, but the secreted form of this molecule is a **pentamer**, held together in an extremely compact form by a **J chain** synthesized by the cell.

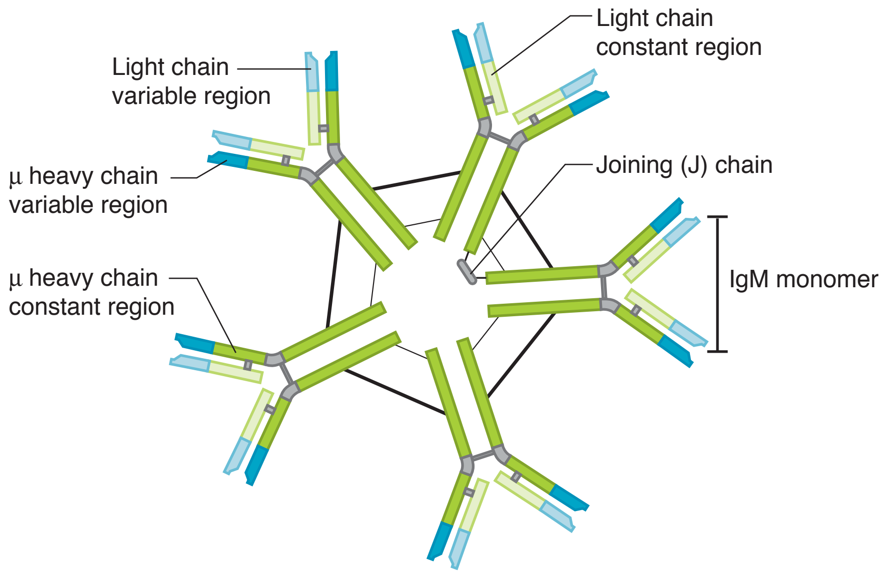


Figure I-7-1. IgM Pentamer

The design of the IgM pentamer maximizes its effect critical to the body early during antigenic challenge. Because of its multimeric structure (5 of the Y-shaped monomers joined into one unit), plasma IgM has 5 times the capacity for binding antigenic epitopes. The valence of the molecule is therefore 10. In other words, 10 identical epitopes can be simultaneously bound, as compared with 2 for the monomeric structure.

This design makes IgM the most effective immunoglobulin isotype at "sponging" the free antigen out of the tissues, and proves critical—as the humoral response evolves—in trapping antigen so it can be presented to the lymphocytes that will ultimately refine the choice of effector mechanism. Although the affinity (binding strength) of the idiotype for the epitope may not be strong early in the immune response, the IgM molecule possesses the highest avidity (number of antigen-binding sites available to bind epitopes) of any immunoglobulin molecule produced in the body.

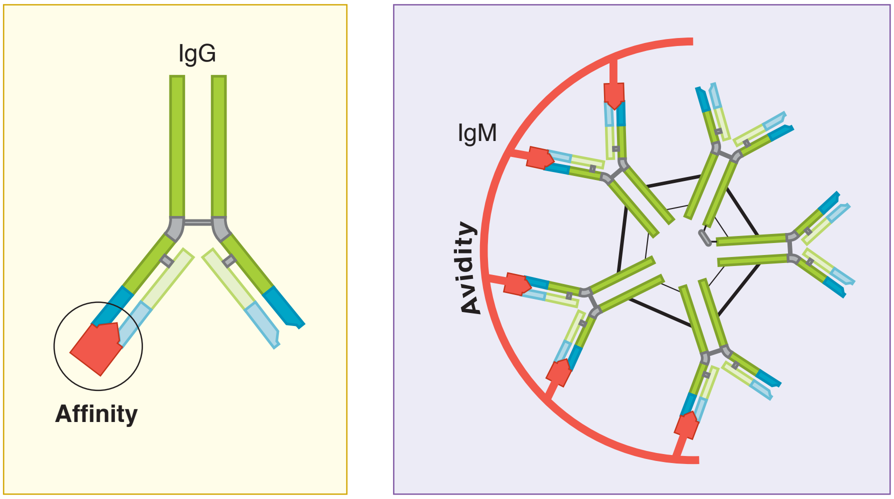


Figure I-7-2. Affinity and Avidity

The multimeric structure of IgM also makes it the most effective antibody at activating complement, a set of serum proteases important in mediating inflammation and antigen removal. Serum IgM is incapable of binding to cellular Fc receptors and thus cannot act as an opsonin (*see* chapter 4) or a mediator of antibody-dependent cell-mediated cytotoxicity (ADCC) (*see* chapter 8).

Clinical Correlate

X-linked hyper-IgM syndrome is characterized by a deficiency of IgG, IgA, and IgE and elevated levels of IgM. IgM levels can reach 2,000 mg/dL (normal 45–250 mg/dL). It is most commonly inherited as an X-linked recessive disorder, but some forms seem to be acquired and can be seen in both sexes.

- Peripheral blood of patients has high numbers of IgM-secreting plasma cells, as well as autoantibodies to neutrophils, platelets, and red blood cells.
- Patients fail to make germinal centers during a humoral immune response.
- Children with this condition suffer recurrent respiratory infections, especially those caused by *Pneumocystis jirovecii*.

The defect in this syndrome is in the gene encoding the CD40 ligand, which maps to the X chromosome. Therefore, Th cells in these patients will fail to express functional CD40L on their membrane, failing to give the costimulatory signal needed for the B-cell response to T-dependent antigens. As a result, only IgM antibodies are produced. The B-cell response to T-independent antigens is unaffected.

ANTIBODIES OF SECONDARY IMMUNE RESPONSES

Class Switching to IgG

The preponderant isotype of immunoglobulin that begins to be produced after IgM during the primary immune response is IgG. IgG is a monomeric molecule with a γ **heavy chain** and a new set of effector functions. A majority of IgG is produced in response to IFN-γ produced by the Th1 cells.

IgG exists in 4 different **subisotypes** (subclasses) in humans, IgG1, IgG2, IgG3, and IgG4, each of which exhibits a slightly different capacity in effector function. In general, however, IgG has the following characteristics:

- Activates complement
- Acts as an opsonin, enhancing phagocytosis
- Neutralizes pathogen and toxins
- Mediates ADCC

IgG is also actively transported across the placenta by receptor-mediated transport and thus plays a crucial role in protection of the fetus during gestation.

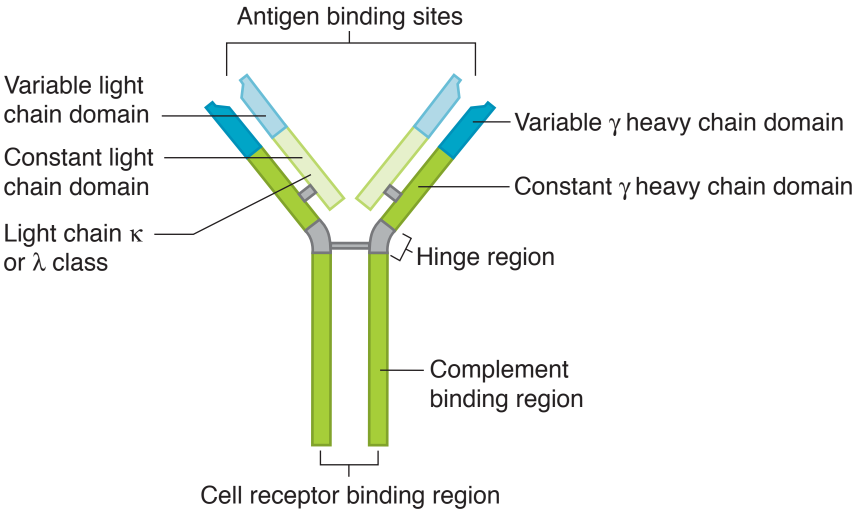


Figure I-7-3. Basic Structure of IgG

Class Switching to IgA

Another isotype of antibody that can be produced following class switching is IgA, though it is more commonly produced in the submucosa than in the lymph nodes and spleen. IgA generally exists as a **dimer**, held together by a J chain similar to that produced with IgM. IgA has the following characteristics:

- Serves as a major protective **defense of the mucosal surfaces of the body**
- Any pathogen that infects the mucosa will induce IgA production by the secretion of TGF-β by infected cells and to a lesser extent, IL-5.
- Functions as a neutralizing antibody by inhibiting the binding of toxins or pathogens to the mucosa of the digestive, respiratory, and urogenital systems (sole function)

- Does not activate complement, act as an opsonin, or mediate ADCC
- Exists in 2 isotypes, IgA1 and IgA2

The **classical pathway** is activated by antigen-antibody complexes and is probably the more phylogenetically advanced system of activation. Both IgG and IgM can activate the system by this pathway, although IgM is the more efficient.

Although the complement cascade is considered a component of the innate immune response, its overlapping stimulation of effector functions of cells of the adaptive immune response, as well as its role in enhancement of inflammation, make it a critical effector system for removal of extracellular invaders and concentration of antigens into the secondary lymphoid organs, where the adaptive immune responses are elicited.

The homing of specific memory cells to epithelial and mucosal surfaces leads to the production of specialized lymphoid aggregations along these barriers. Collectively referred to as **mucosal-associated lymphoid tissues** (MALT), they include the tonsils and Peyer patches, as well as numerous, less well-organized lymphoid accumulations in the lamina propria. Th2 cells in these sites are dedicated to providing help for class switching to IgA. Most IgA-secreting B lymphocytes and plasma cells in the body will be found in these locations.

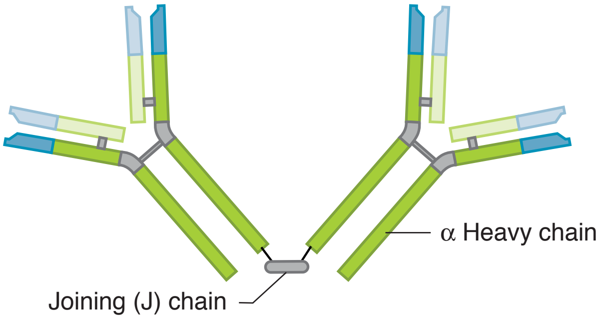


Figure I-7-4. The IgA Dimer

Secretory IgA (that which is released across the mucosa of the respiratory, digestive, and urogenital tracts) differs from serum IgA in an important fashion. As the IgA dimer is produced by plasma cells and B lymphocytes, it becomes bound to poly-Ig receptors on the basolateral side of the epithelia, is endocytosed, and is released into the lumen bound to a secretory piece that is the residue of the receptor. The **secretory component** thus serves an important function in transepithelial transport, and once in the lumen of the tract, has a function in protecting the molecule from proteolytic cleavage.

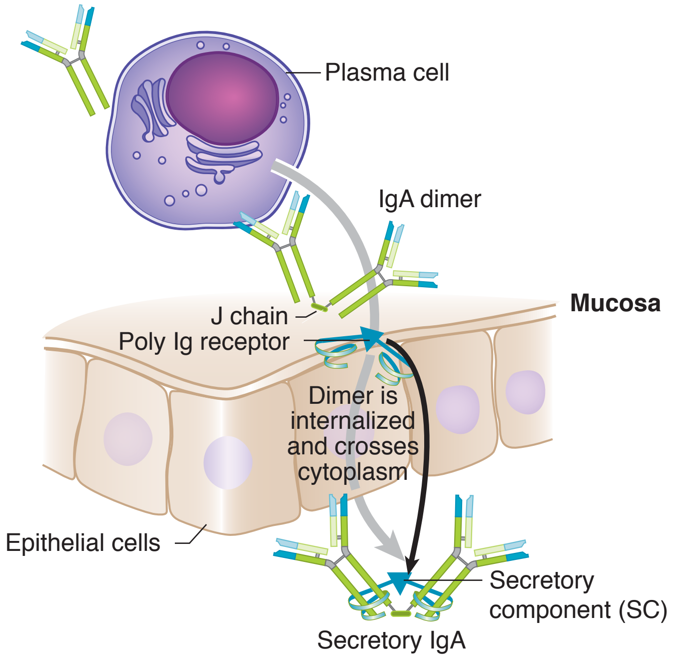


Figure I-7-5. Secretory IgA

Class Switching to IgE

IgE binds directly to Fcε receptors present on mast cells, eosinophils and basophils, and is involved in elicitation of protective immune responses against parasites and allergens (*see* chapter 12). It does not activate complement or act as an opsonin. Its heavy chain is called the ε chain.

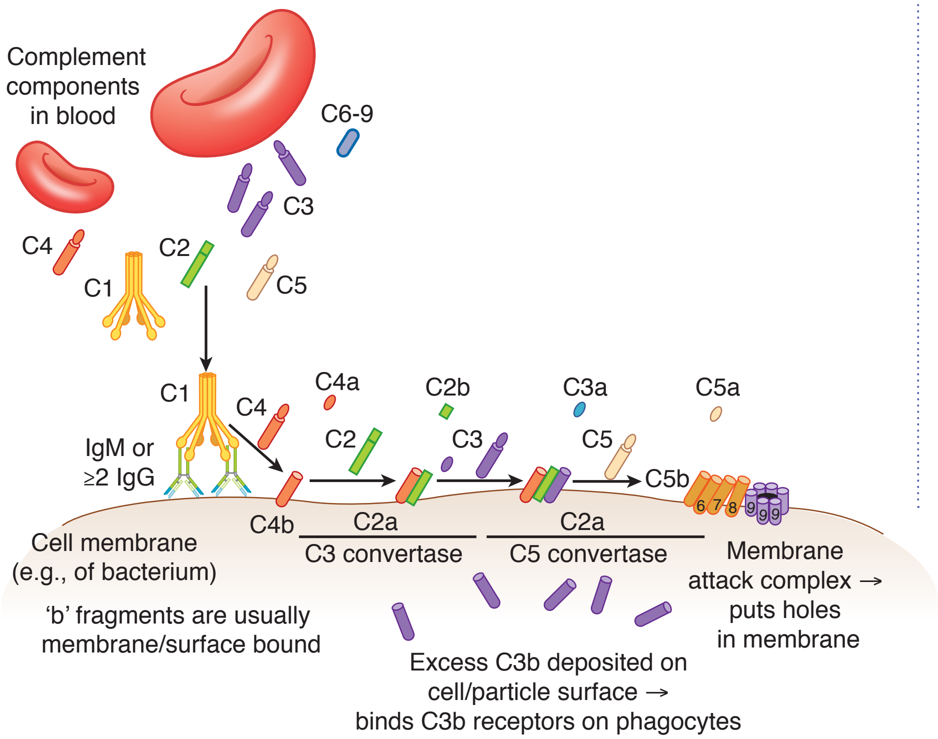


Figure I-7-6. Classical Complement Pathway

Table I-7-1. Biologic Functions of the Antibody Isotypes

	IgM	IgG	IgA	IgD	IgE
Heavy chain	μ	γ	α	δ	ε
Adult serum levels (in mg/dL)	45–250	620–1,400	80–350	Trace	Trace
Functions					
Complement activation, classic pathway	+	+	–	–	–
Neutralization	+/–	+	+	–	–
Opsonization	–	+	–	–	–
Antibody-dependent cell-mediated cytotoxicity (ADCC)	–	+	–	–	+/–
Placental transport	–	+	–	–	–
Naive B-cell antigen receptor	+	–	–	+	–
Memory B-cell antigen receptor (one only)	–	+	+	–	+
Trigger mast cell granule release	–	–	–	–	+

Clinical Correlate

Immunodeficiencies Involving B Lymphocytes

Patients with B-cell deficiencies usually present with recurrent pyogenic infections with extracellular pathogens. The absence of immunoglobulins for opsonization and complement activation is a major problem (*see* chapter 11).

- T-cell immune system is intact.
- T-cell activities against intracellular pathogens, delayed-type hypersensitivity, and tumor rejection are normal (*see* chapter 8).

Cell-Mediated Immunity

8

Learning Objectives

- ❑ Describe the role of macrophages, B cells, cytotoxic T lymphocytes, and NK cells
- ❑ Demonstrate understanding of antibody dependent cell-mediated cytotoxicity
- ❑ Demonstrate understanding of agglutination
- ❑ Answer questions about ABO testing
- ❑ Demonstrate understanding of lab tests, including labeled antibody systems, fluorescent antibody tests, enzyme-linked immunosorbent assay, and fluorescence activated cell sorting

CELL-MEDIATED IMMUNITY

Cell-mediated immunity has evolved to battle 2 different types of pathogens:

- **Facultative intracellular pathogens**, which have adapted to living inside of phagocytic cells that are designed to kill them
- **Obligate intracellular pathogens**, which can't replicate outside of host cells

Cell-mediated immunity is dictated by the Th1 response and is mediated primarily via macrophages and CD8+ T cells. While the Th1 response is geared toward eliminating intracellular pathogens, Th cells—in general—direct all aspects of the immune system.

The primary mechanism by which Th cells direct all aspects of immunity is the secretion of cytokines. NK cells also have a role in this type of immunity; they were covered in chapter 4 and will be reviewed here.

MACROPHAGES/B CELLS

The Th1 response activates both macrophages and B cells via the cytokine IFN-γ. IFN-γ activates classical M1 macrophages to eradicate intracellular pathogens and induces B cells to class switch to produce opsonizing IgG antibodies that can assist the macrophages with phagocytosis.

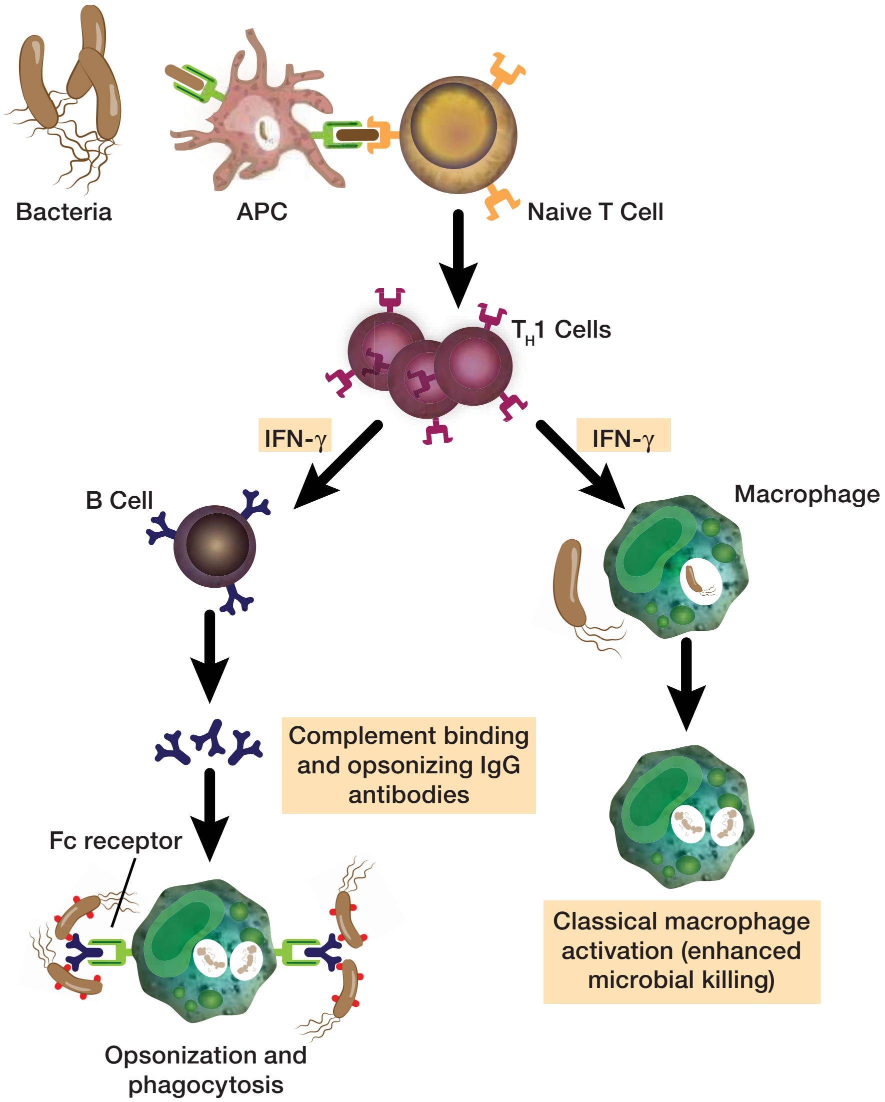


Figure I-8-1. Cell-Mediated Immunity

Macrophage-Th Cell Interaction

The binding of the TCR of the naive Th cell to the MHC class II–peptide complex of the macrophage provides the first signal to the T cell to begin its activation. This provides the antigenic specificity of the response. Co-stimulatory molecules on macrophage provide the second signal, and cytokines secreted by the macrophage and the activating T cells themselves induce the proliferation (clonal expansion) and differentiation of the T cells into effector cells and memory cells. Effector cells leave the secondary lymphoid tissue, enter into circulation, and travel to the site of the infection.

Table I-8-1. Summary of Macrophage Molecules and Function

Cell	CD Markers	MHC class I	Effector Mechanisms
Macrophage	CD14 (LPS receptor)	yes	Nitric oxide, oxygen radicals, TNF-α

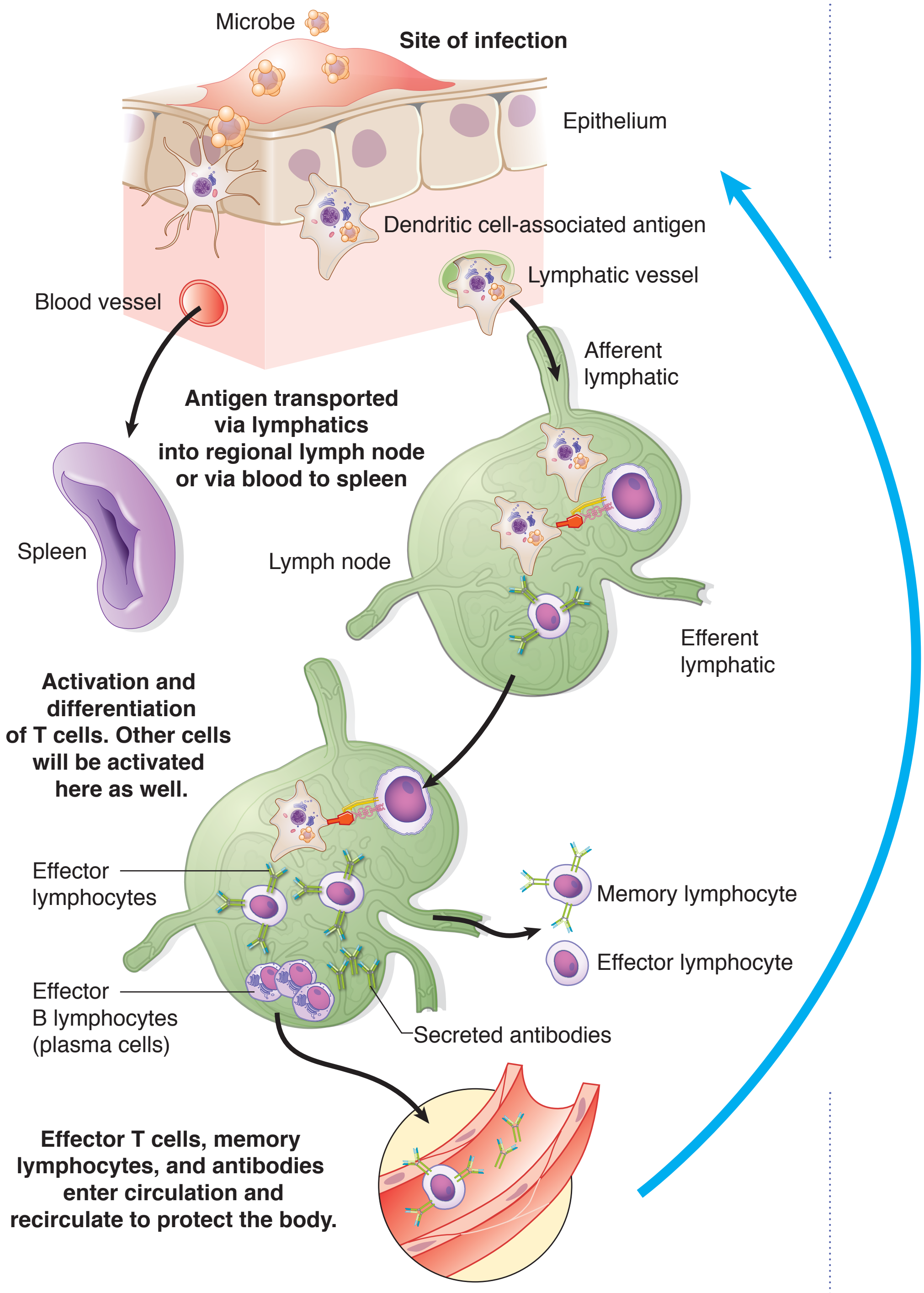


Figure I-8-2. Migration of Effector Cells to the Site of Infection

The proliferation of naive T cells in response to antigen recognition is mediated principally by an autocrine growth pathway, in which the responding T cell secretes its own growth-promoting cytokines and also expresses receptor molecules for these factors. IL-2 is the most important growth factor for T cells and stimulates the proliferation of clones of T cells specific to that antigen. Additionally, the T cells provide IFN-γ, which promotes macrophage activation that also helps to activate Th cells. The production of IL-12 by the macrophage also helps to activate the Th cells. Together, IL-12 and IFN-γ also help to promote the differentiation of the naïve Th cell into a Th1 cell.

The reaction mediated by the Th1 cell via macrophage and CD8+ T cell activation is often referred to as the delayed-type hypersensitivity (DTH) reaction. Although this is the normal response of the body to intracellular pathogens, it is the exact same mechanism of cellular interactions and cytokine production as a hypersensitivity to poison ivy or nickel (*see* chapter 12).

CYTOTOXIC T LYMPHOCYTES (CTLS)

CTLs recognize the cell they will ultimately kill by interaction between their TCR and the MHC class I peptide complex on the surface of the target cell.

- If the cell in question is performing **normal functions** and therefore producing normal "self" peptides, there should be no CD8+ T cells that have a complementary TCR structure.
- If the cell is **infected with an intracellular pathogen or is expressing neoantigens reflective of tumor transformation**, some small proportion of those CD8+ T cells generated from the thymus should be capable of binding their TCRs to this MHC class I/non-self-peptide complex.

Unfortunately, because of the extreme polymorphism of the HLA (MHC) system in humans, when tissues are transplanted between nonidentical individuals, cells of the transplant are often targeted by CTLs as abnormal. In spite of the fact that they may only be presenting normal cellular peptides, in these cases the HLA molecules themselves are different enough to elicit an immune response (*see* chapter 13). CTLs are capable of differentiation and cloning by themselves in the presence of the appropriate MHC class I non-self peptide complex stimulus, but are much more effective in so doing if they are assisted by signals from Th1 cells. The Th1 cell secretes IL-2, which acts on CD8+ cells to enhance their differentiation and cloning. This occurs via cross priming as discussed in chapter 5. Additionally, interferons produced in the area will increase the expression of MHC molecules to make target cells more susceptible to killing.

CTLs kill their target cells by the delivery of toxic granule contents that induce the apoptosis of the cell to which they attach. This process occurs in 4 phases:

- Attachment to the target cell (mediated by TCR, CD8, and LFA-1 integrin)
- Activation (cytoskeletal rearrangement to concentrate granules against attached target cell)
- Exocytosis of granule contents (perforin and granzymes)
- Detachment from the target cell

The death of the target cell may be mediated in distinct fashions. First, perforin present in the CTL granules creates pores in the membrane of the target cell through which granzymes (serine proteases) enter the cell, inducing the activation of caspases, which activate the "death domain." Second, cytokines such as IFN-γ with TNF-α or TNF-β can induce apoptosis. Furthermore, activated CTLs express a membrane protein called Fas ligand (FasL), which may bind to its complementary structure, Fas, on the target cell. When this occurs, caspases are induced and death results.

Table I-8-2. Summary of Cytotoxic T-Cell Markers and Function

Cell	CD Markers	MHC class I	Effector Mechanisms
CTL	CD8, CD3, TCR, CD2	Yes	Perforin, granzymes, cytokines

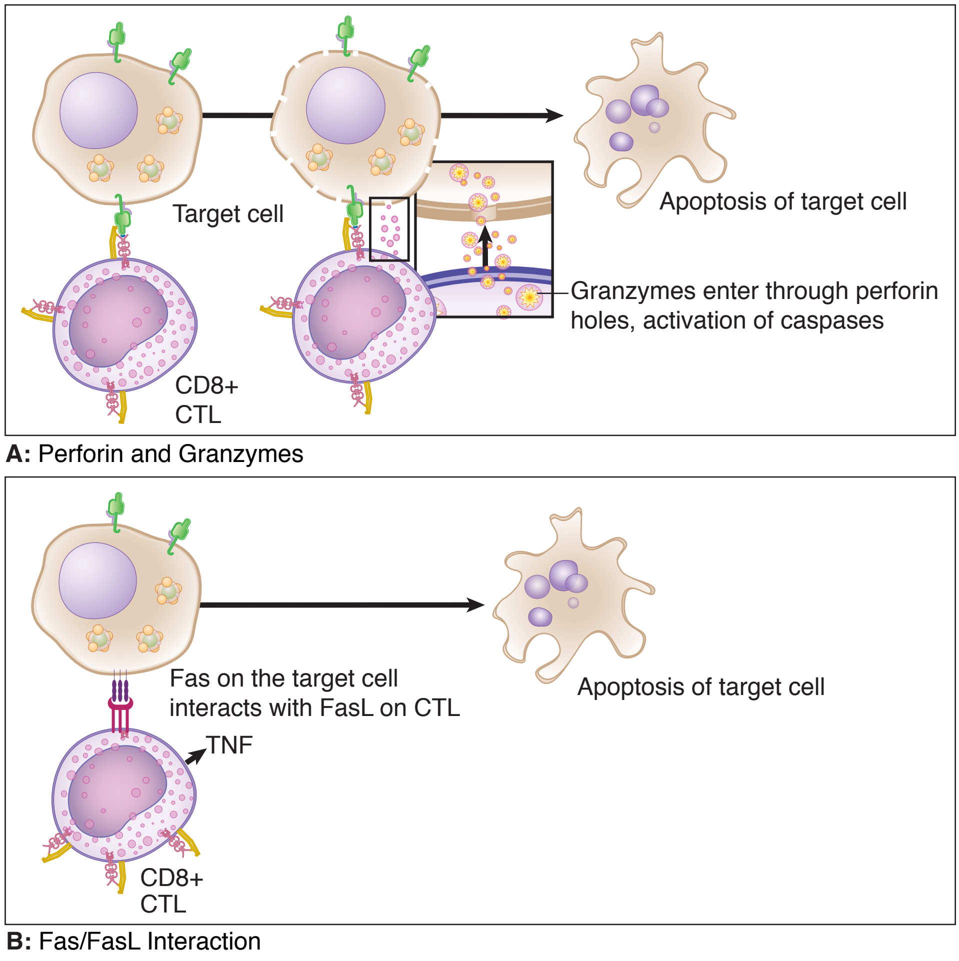


Figure I-8-3. Mechanisms of Cytotoxic T-Cell Killing

NK CELLS

Another cell-mediated effector mechanism enhanced by the action of Th1 cells is NK cell-killing. Since the innate function of NK cells was discussed in chapter 4, the table below summarizes that information.

Table I-8-3. Summary of NK Cell Markers and Function

Cell	CD Markers	MHC class I	Effector Mechanisms
NK	CD16 (FcR)* CD56 (CAM)	Inhibited by the normal expression of class I MHC via HLA-E.	Perforin, granzymes, cytokines (identical to CTL)**

*Keep in mind that CD16 is an FcR and is present on cells other than NK cells.

**The effector mechanisms of NK cell killing are identical to CTLs; the only difference between them is how they recognize the antigen.

ADCC

A final mechanism of cytotoxicity which bridges humoral and cell-mediated effector systems in the body is antibody-dependent cell-mediated cytotoxicity

(ADCC). A number of cells with cytotoxic potential (NK cells, macrophages, neutrophils, and eosinophils) have membrane receptors for the Fc region of IgG (aka CD16). When IgG is specifically bound to a target cell, the cytotoxic cells can bind to the free Fc "tail" and subsequently cause lysis of the target cell.

Although these effectors are not specific for antigen, the specificity of the idiotype of the antibody directs their cytotoxicity. The mechanism of target cell killing in these cases may involve the following:

- Lytic enzymes
- Tumor necrosis factor
- Perforin/granzymes

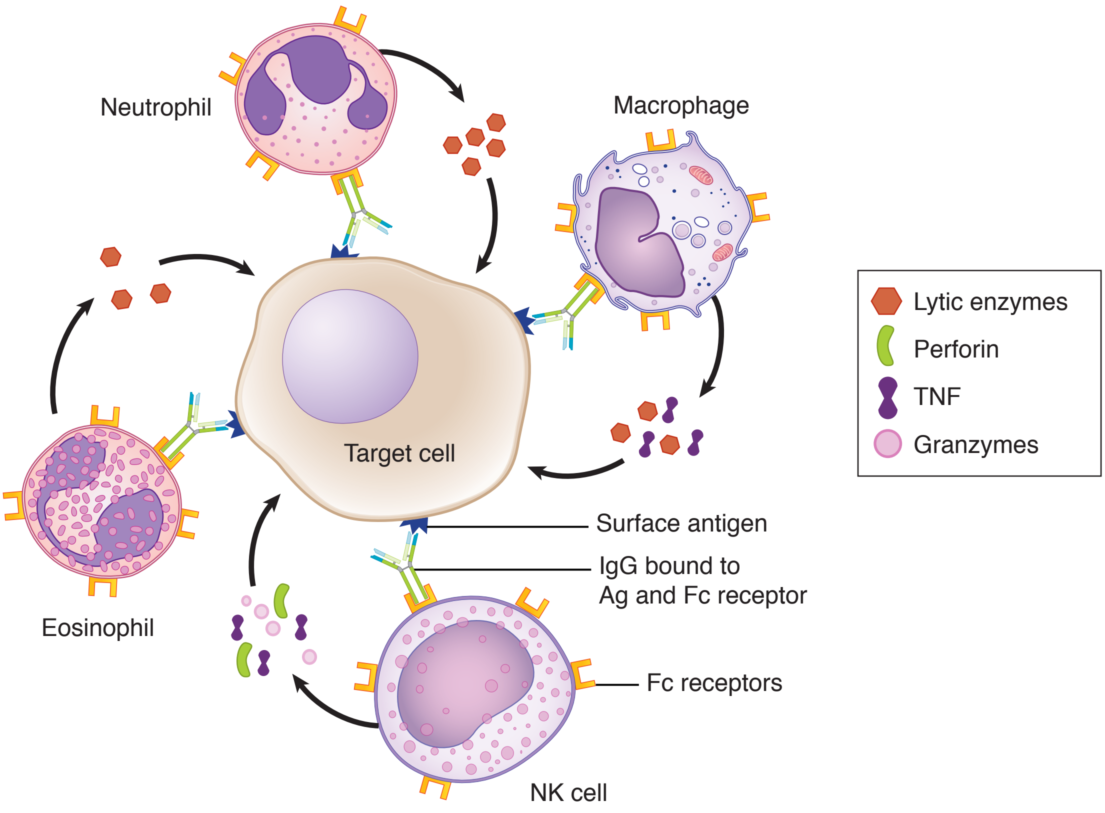


Figure I-8-4. Antibody-Dependent Cell-Mediated Cytotoxicity

Immunodiagnostics

9

Learning Objectives

- ❏ Describe the recirculation pattern of memory B and T lymphocytes
- ❏ Differentiate between primary and secondary immune response

SEROLOGY

Serology is an important diagnostic tool for many diseases including infections and autoimmune disorders. The interaction of antigen and antibody that occurs in vivo and in clinical laboratory settings provides the basis for all serologically based tests.

IgM and IgG

IgM is the principal immunoglobulin of the primary immune response when antigen is first encountered. It is replaced in later responses by antibodies of different isotypes, mostly IgG in the serum. Although IgM antibodies are occasionally produced at low levels during secondary and later immunologic responses, they are always produced by cells encountering that antigen for the first time.

IgM is extremely important in diagnosis of recent infections and infections in neonates or fetuses. For example, a patient with IgM antibodies to the core antigen of HBV (HBcAb) is an important diagnostic tool because it suggests a recent or acute infection and may also be found in the window period when other antibodies may not be detectable.

Also, we can make certain assumptions based on serology using IgM in the diagnoses of neonatal or fetal infections. For example, a neonate that is making IgM specific for a virus such as rubella is infected with the virus rather than immune or protected by maternal antibodies. This is because IgM does not cross the placenta. Therefore, the only way a neonate or fetus can be producing IgM specific for a certain pathogen is if the neonate or fetus were infected with that agent.

The predominant isotype of immunoglobulin that begins to be produced after IgM during the primary immune response is IgG.

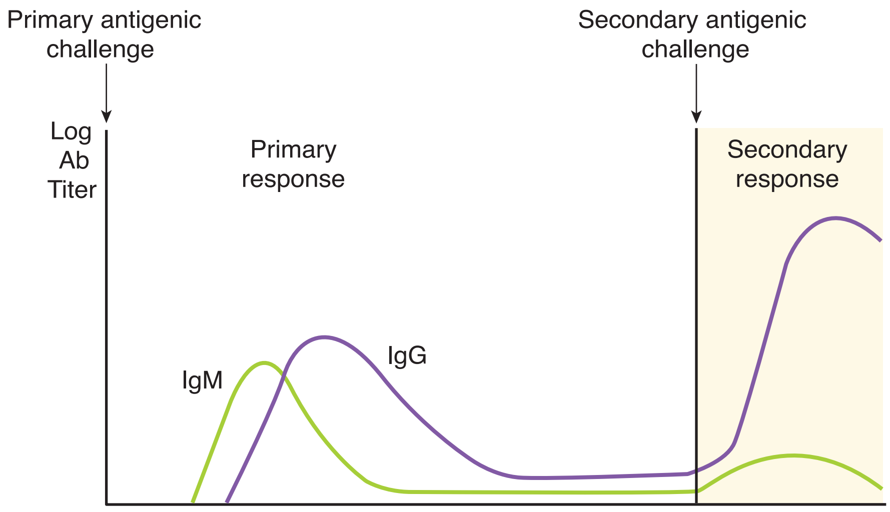


Figure I-9-1. Primary and Secondary Antibody Responses

Ideotype, Isotype, and Allotype

The unique pocket created by the variable regions of the light chain and the heavy chain is called the **idiotype** of the antibody. It is the region that is specific for antigen. It is both extremely diverse and specific. Each individual is capable of producing hundreds of millions of unique idiotypes.

The **isotype** of the antibody is determined by the constant region and is encoded by the heavy chain genes. The isotype of the antibody determines its function.

The **allotype** of an antibody is an allelic difference in the same antibody isotypes that differ between people. For example, 2 individuals with the same IgG have subtle differences in their immunoglobulins due to heterogeneity which tends to be specific for individuals. A patient receiving pooled gamma globulins might react to these allotypic differences in the constant regions which may result in type III hypersensitivity reactions.

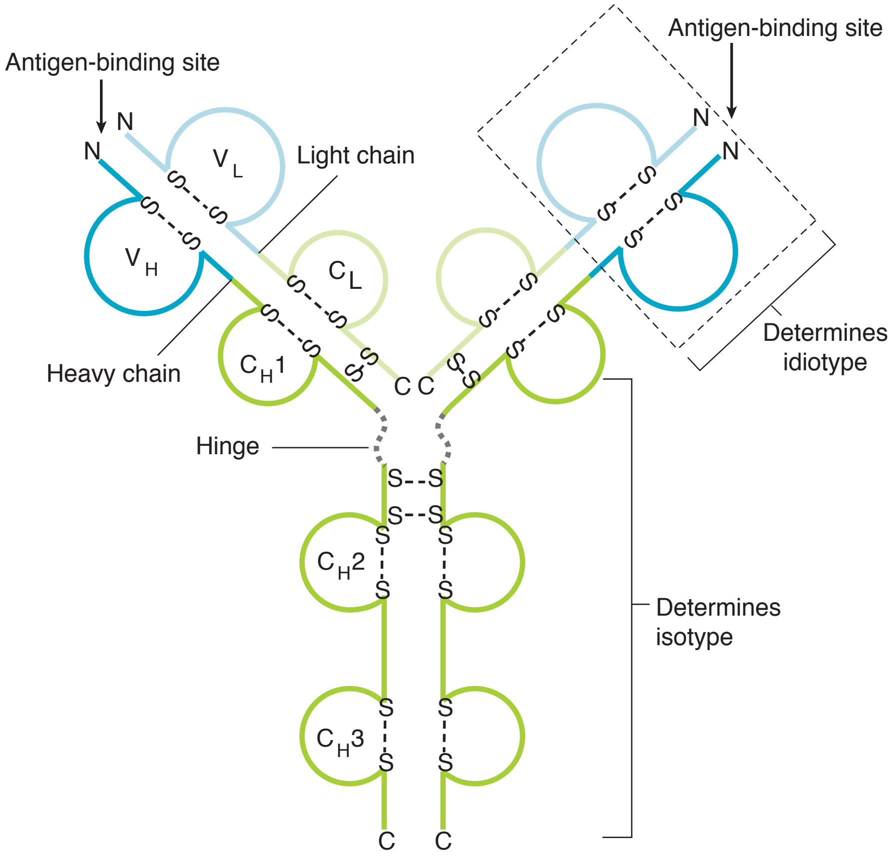


Figure I-9-2. B-Lymphocyte Antigen Recognition Molecule (Membrane-Bound Immunoglobulin)

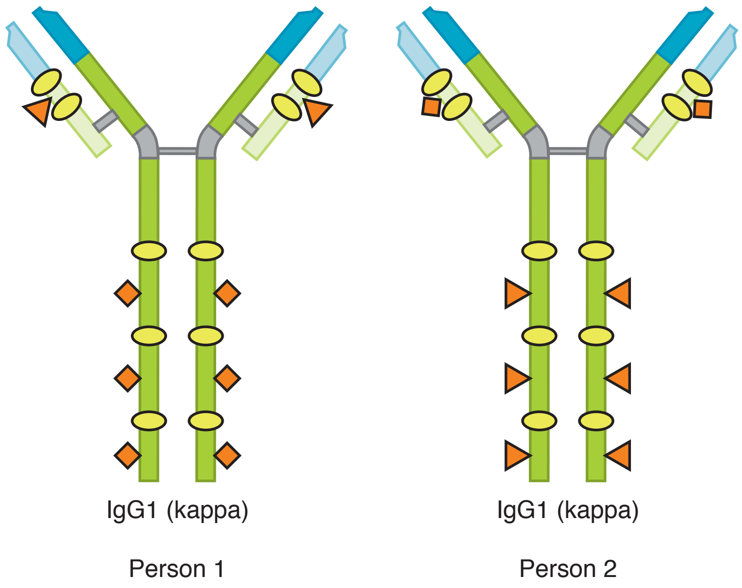


Figure I-9-3. Allotypes

Papain versus Pepsin Digestion

The biologic function of segments of the antibody molecule was first elucidated by digestion of these molecules with proteolytic enzymes. If an antibody molecule is digested with papain, cleavage occurs above the disulfide bonds that hold the heavy chains together. This generates 3 separate fragments, two of which are called Fab (fragment antigen binding), and one of which is called Fc (fragment crystallizable).

Cleavage of the antibody molecule with pepsin generates one large fragment called F(ab´)2 and a digested Fc fragment. The bridging of antigens by antibody molecules is required for agglutination of particulate antigens or the precipitation of soluble antigens.

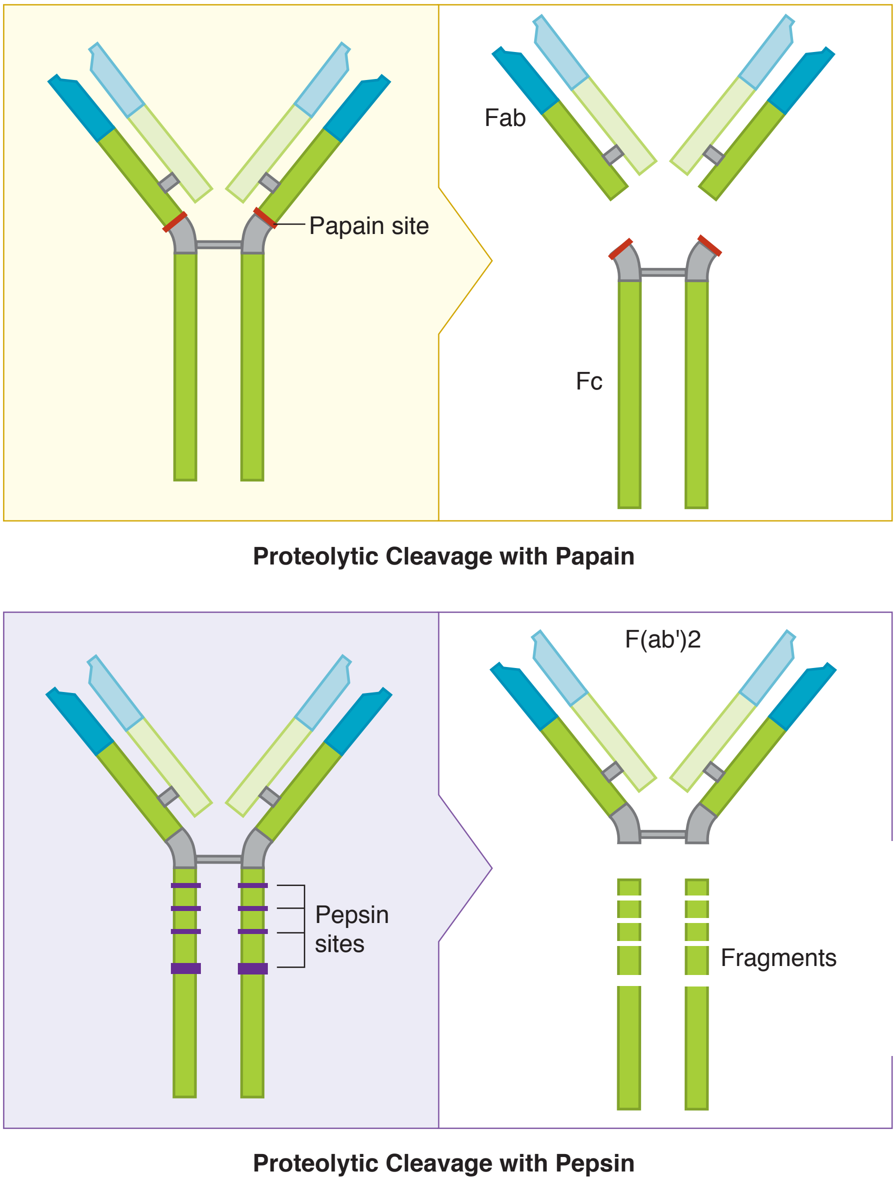


Figure I-9-4. Proteolytic Cleavage of Immunoglobulin by Papain/Pepsin

Zone of Equivalence

Interaction of antigen and antibody occurs in vivo, and in clinical settings it provides the basis for all serologically based assays. The formation of immune complexes produces a visible reaction that is the basis of precipitation and agglutination assays. Agglutination and precipitation are maximized when multiple antibody molecules share the binding of multiple antigenic determinants, a condition known as **equivalence**.

In vivo, the precipitation of such complexes from the blood is critical to the trapping of pathogens and the initiation of the immune response in the secondary lymphoid organs, as well as the initiation of the pathologic phase of many immune complex-mediated diseases. In vitro, the kinetics of such reactions can be observed by titration of antigen against its specific antibody.

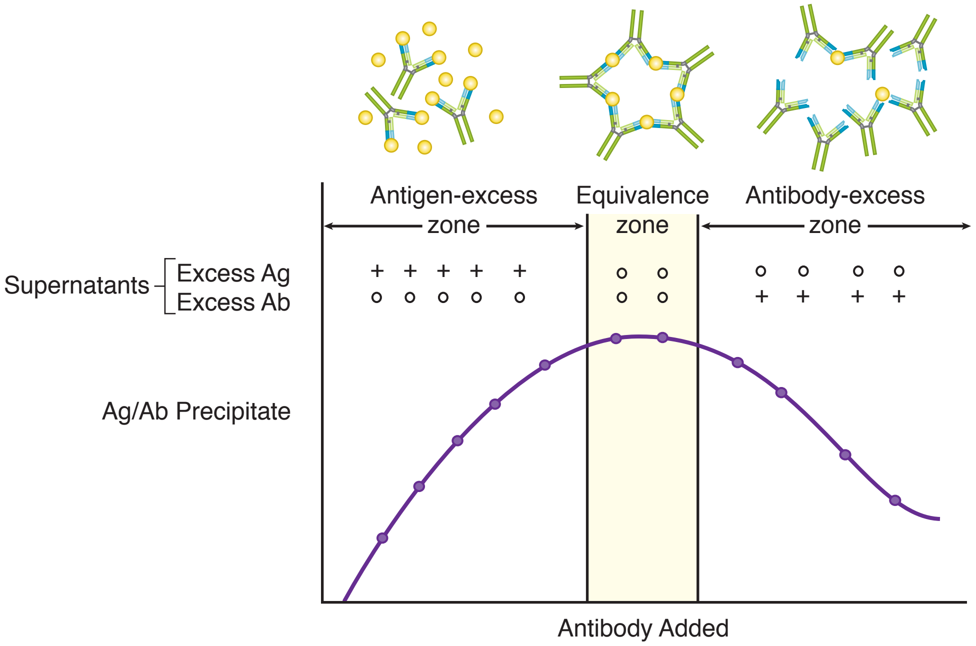


Figure I-9-5. Precipitation of Ag–Ab Complexes during Titration of Ag with Ab

Figure I-9-5 demonstrates the normal progression of the antibody response during many infectious diseases.

- At the **start of the infection**, the patient is in a state of antigen excess; the pathogen is proliferating in the host and the development of specific antibodies has not yet begun.
- As the patient begins to make an **adequate antibody response**, he enters the equivalence zone; all available antigen is complexed with antibody, and neither free antigen nor free antibody can be detected in the serum.
- Finally, as the **infection is resolved**, the patient enters the antibody excess zone, when more antibody is being produced than is necessary to precipitate all available antigen.

The clinical demonstration of this phenomenon is most easily seen in our use of the serologic diagnosis of hepatitis B infection.

- Early in the course of this infection, HBsAg is easily detectable in the blood. The patient is in the antigen excess zone for this antigen.

- As the patient enters the window period (the equivalence zone), the HBsAg disappears from the circulation because it is being removed by antibody precipitation.
- Finally, antibody titers (HBsAb) rise in the serum as the patient enters the antibody excess zone and resolves the infection.

Although the "window period" in the hepatitis B infection is used exclusively to note the absence of HBsAg and HBsAb from the serum (only antigen–antibody response that has a clinical significance in the prognosis of disease). An equivalence zone is a universal stage in the development of any antibody–antigen interaction.

Monoclonal versus Polyclonal Antiserum

Polyclonal antiserum is generally produced in an individual naturally during any type of infection. It represents many different clones of B cells that are making antibodies to many different epitopes on an antigen; therefore, a heterogenous complex mixture of antibodies is produced. Alternatively, polyclonal antiserum can be produced by inoculating an animal such as a mouse, rabbit or goat. This is done to produce commercial antiserum that can be purchased and utilized in laboratories.

Monoclonal antibodies are produced by one clone of B cells with specificity for the exact same epitope on an antigen. Monoclonal antibodies are produced in the laboratory and are used in all aspects of medicine from diagnostics to treatments for various types of cancer and autoimmune diseases.

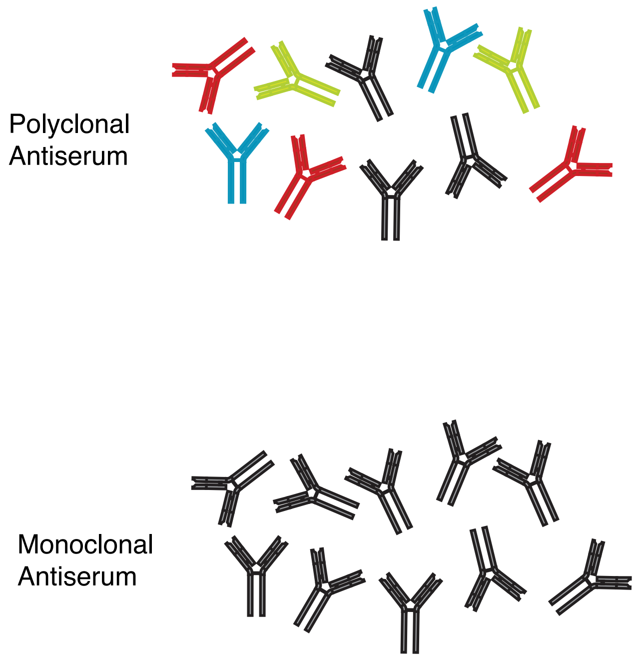


Figure I-9-6. Polyclonal versus Monoclonal Antibodies

Direct versus Indirect Serologic Tests

Direct serologic testing utilizes a known antiserum in order to detect an unknown antigen, either foreign or self. Direct tests are qualitative and provide results relatively quickly. They are used mostly for screening purposes.

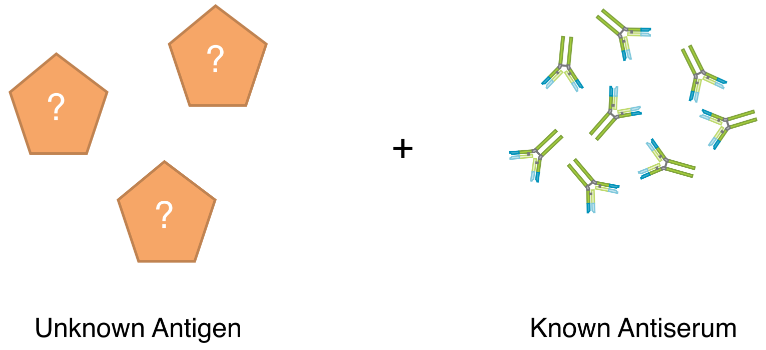


Figure I-9-7. Direct Serologic Test

Indirect serologic testing utilizes antibodies from the patient that may be specific for either self or foreign antigen. This test is based on the concept that antibodies are produced in response to a specific disease state. Indirect tests may be qualitative and used for screening purposes or quantitative, which provides the amount of antibody in the patient's serum. Quantitative tests are also called antibody titers. A titer is often done to follow the progression of disease in a patient by looking for an increase or decrease in the level of antibodies. A titer involves diluting the patient's serum out to see how dilute the serum can be and still detect antigens in a solution.

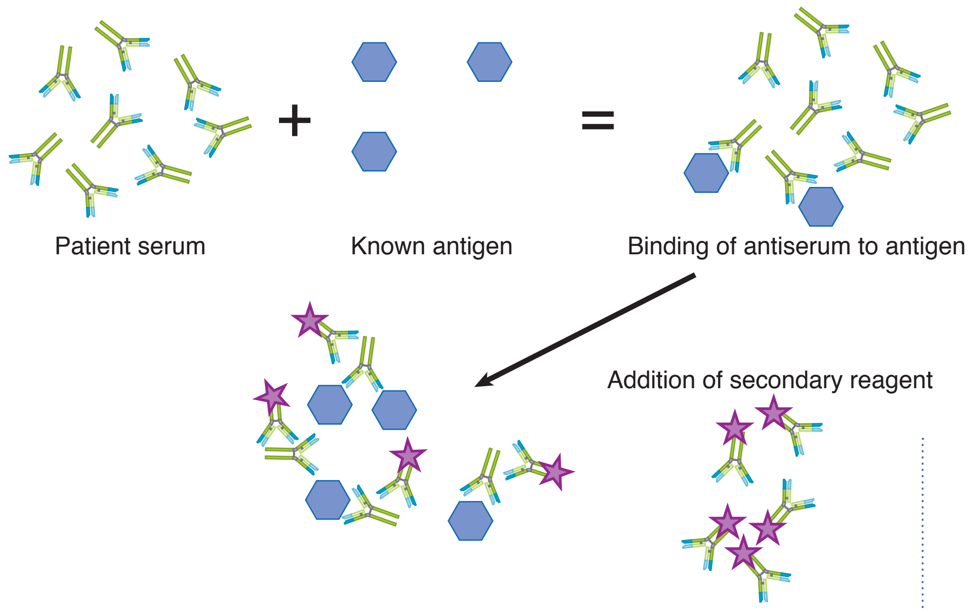


Figure I-9-8. Indirect Serologic Test

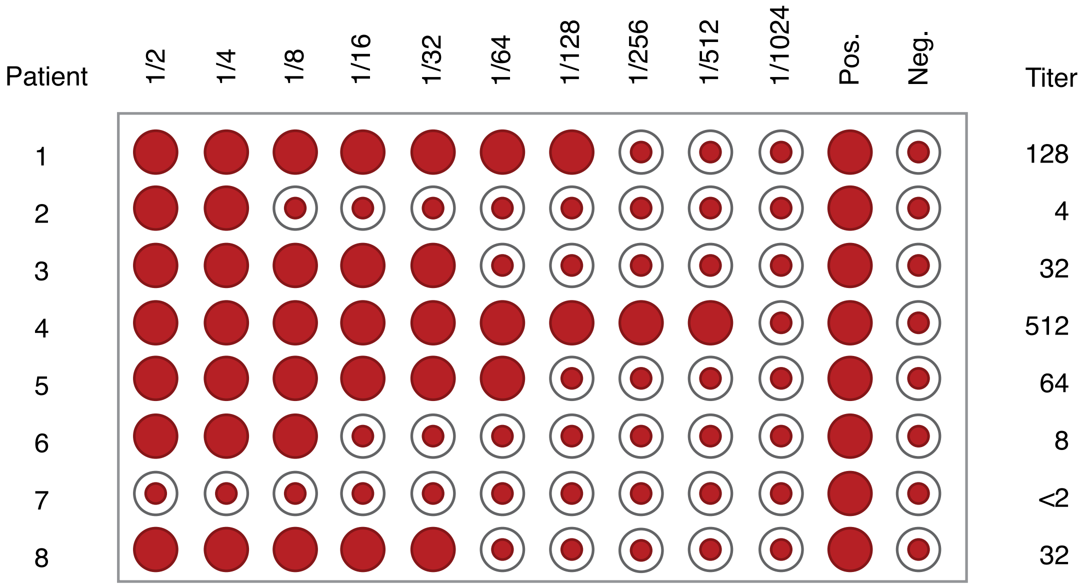


Figure I-9-9. Antibody Titers

Most immunologic tests can be performed using direct or indirect measures. Indirect tests are generally more specific, resulting in fewer false-positives. The Coombs, ELISA, and fluorescent antibody tests are all examples of tests that can be utilized in either a direct or indirect manner.

AGGLUTINATION

Agglutination tests are widespread in clinical medicine and are simply a variation on precipitation reactions. In agglutination reactions, the antigen is a particulate antigen such as RBCs or latex beads. Both will clump up to form of a lattice of antibody-bound particles in the presence of appropriate antibodies.

- Latex bead agglutination tests are available for the diagnosis of cerebrospinal infections such as *Haemophilus, pneumococcus, meningococcus, and Cryptococcus*. In each of these cases, antibodies against these organisms are conjugated to latex beads, and the presence of microbial antigens in the CSF is detected by the subsequent agglutination of those beads.
- RBC agglutination reactions are important in defining ABO blood groups, diagnosing Epstein-Barr virus infection (the monospot test), and identifying Coombs test for Rh incompatibility.

Coombs Test

Two variations of the Coombs test exist. The **direct Coombs** is designed to identify maternal anti-Rh antibodies that are already bound to infant RBCs or antibodies bound to RBCs in patients with autoimmune hemolytic anemia.

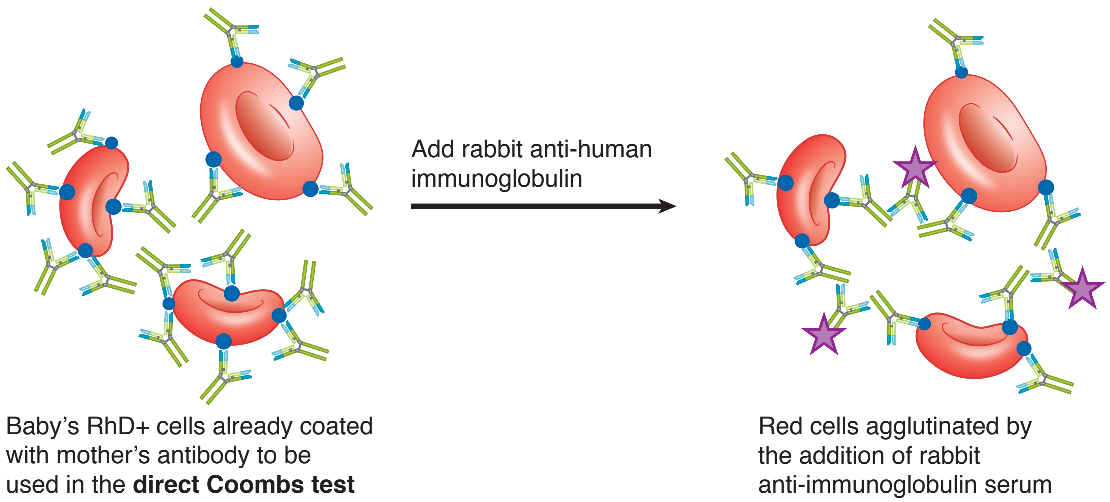


Figure I-9-10. Direct Coombs Test

The **indirect Coombs test** is designed to identify Rh-negative mothers who are producing anti-Rh antibodies of the IgG isotype, which may be transferred across the placenta harming Rh-positive fetuses. The indirect Coombs is also used in the diagnosis of transfusion reactions.

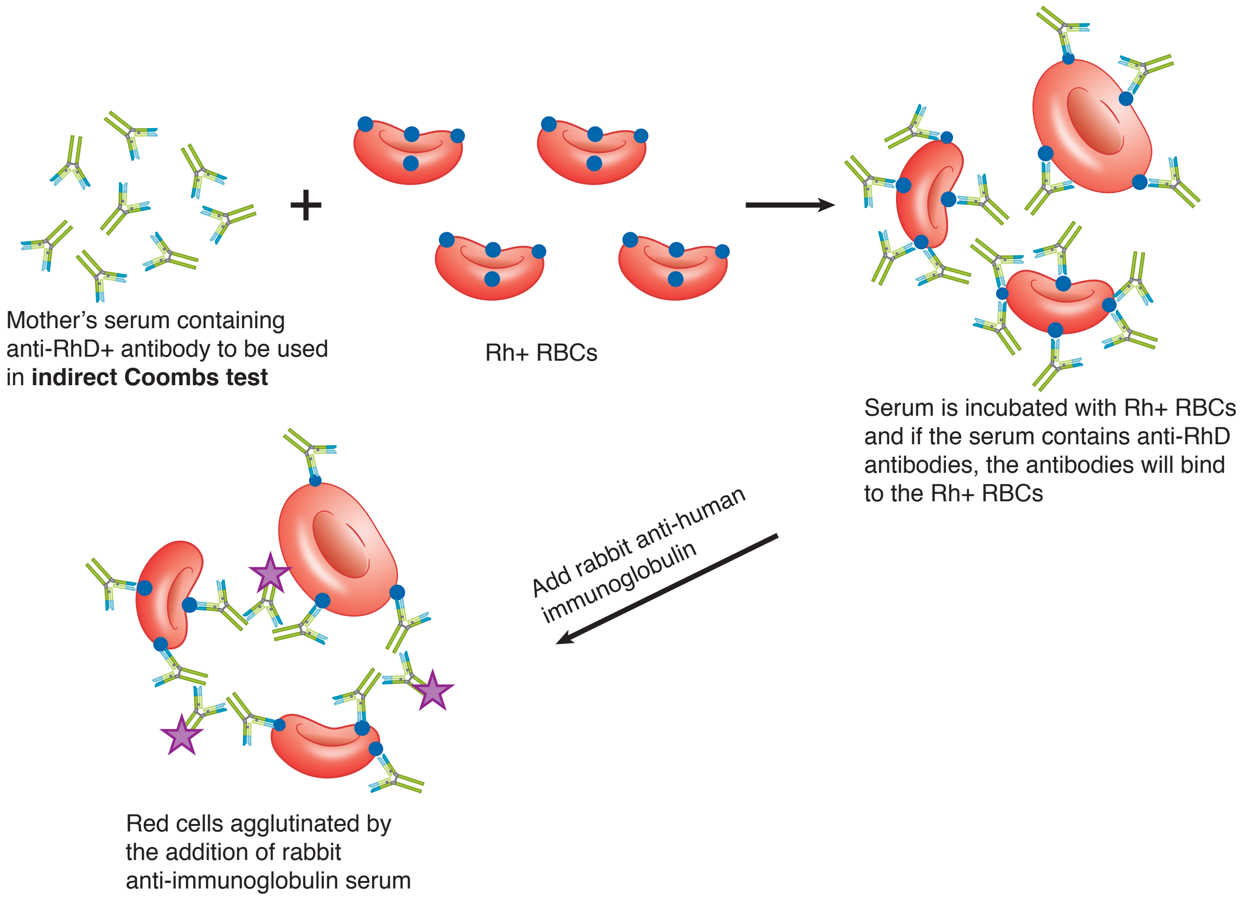


Figure I-9-11. Indirect Coombs Test

ABO TESTING

ABO blood typing is a uniform first step in all tissue transplantation because ABO incompatibilities will cause hyperacute graft rejection in the host. The ABO blood group antigens are a group of glycoprotein molecules expressed on the surface of erythrocytes and endothelial cells. Natural isohemagglutinins (IgM antibodies that will agglutinate the glycoprotein molecules on the red blood cells of nonidentical individuals) are produced in response to similar molecules expressed on the intestinal normal flora. A person is protected by self-tolerance from producing antibodies that would agglutinate his own red blood cells, but will produce those agglutinins that will react with the red blood cells from other individuals.

A: agglutination

N: no agglutination

		Blood Types: A	B	O	AB
Serum from: Contains:	A Anti-B Antibodies	N	A	N	A
Serum from: Contains:	B Anti-A Antibodies	A	N	N	A
Serum from: Contains:	O Anti-A and Anti-B Antibodies	A	A	N	A
Serum from: Contains:	AB No Antibodies to A or B	N	N	N	N

Figure I-9-12. Agglutination Test for Blood Typing

LABELED ANTIBODY SYSTEMS

Labeled antibody systems are utilized for the detection of antigens, which may be either self or foreign. These antigens can be visualized using a combination of specific antibody that is labeled or tagged with a compound used for its detection. Common tags include fluorescent compounds and enzymes.

Each of the following discussed is an example of a labeled antibody system. Additionally, fluorescent antibody tests and ELISAs can be done using either direct or indirect tests as described previously.

Fluorescent Antibody Tests

The **direct fluorescent antibody test (DFA)** is used to detect and localize antigen in the patient. The tissue sample to be tested is treated with antibodies against that particular antigen that have been labeled with a fluorescent dye. If the antigen is present in the tissues, the fluorescent-labeled antibodies will bind, and their binding can be detected with a microscope. Variations of this test are used to diagnose respiratory syncytial virus, herpes simplex 1 and 2, rabies in animal tissues, and *Pneumocystis* infections.

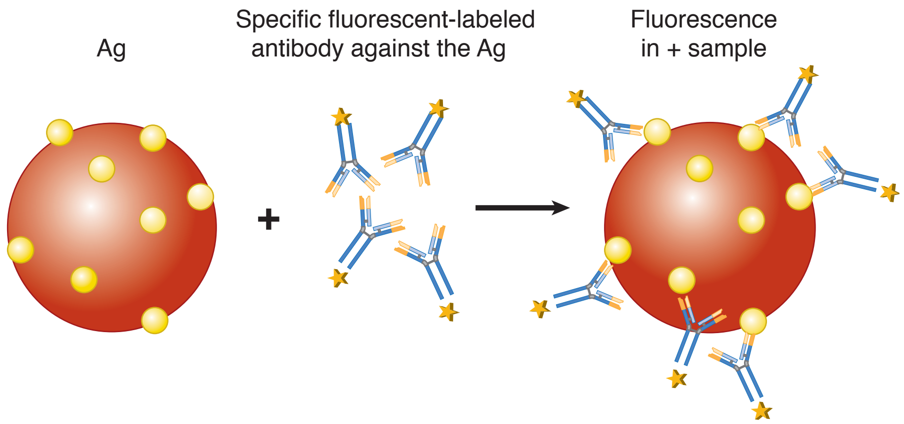


Figure I-9-13. Direct Fluorescent Antibody (DFA) Test

The **indirect fluorescent antibody test (IFA)** is used to detect pathogen-specific antibodies in the patient. In this case, a laboratory-generated sample of infected tissue is mixed with serum from the patient. A fluorescent labeled anti-immunoglobulin is then added. If binding of antibodies from the patient to the tissue sample occurs, then the fluorescent antibodies can be bound and detected by microscopy. This technique can be used to detect autoantibodies in various autoimmune diseases.

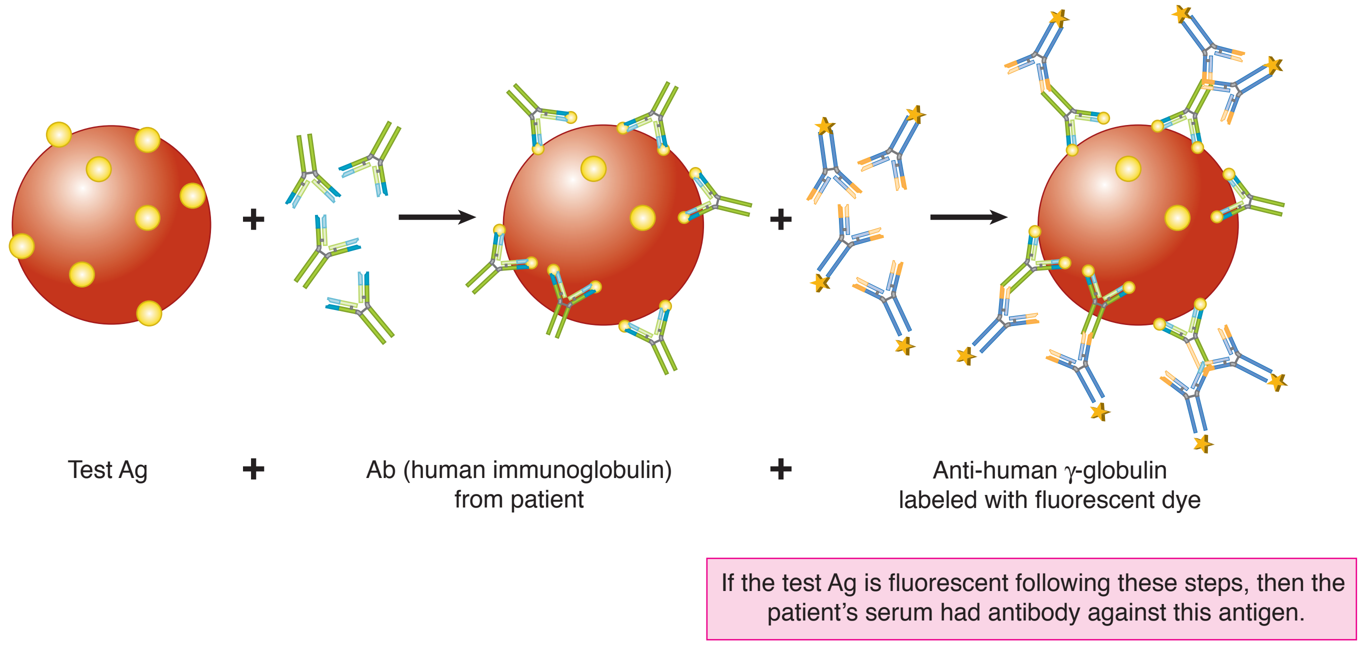


Figure I-9-14. Indirect Fluorescent Antibody (IFA) Test

Enzyme-linked Immunosorbent Assay (ELISA)

The ELISA is an extremely sensitive test (as little as 10^{-9} g of material can be detected). It can be used to detect the presence of hormones, drugs, antibiotics, serum proteins, infectious disease antigens, and tumor markers. It does so by utilizing a chromogenic substrate that undergoes an enzyme-mediated color change.

In the screening test for HIV infection, the ELISA is used with the p24 capsid antigen coated onto microtiter plates. The serum from the patient is then added, followed by addition of an enzyme-labeled antihuman immunoglobulin. Finally, the chromogenic substrate is added, and the production of a color change in the well can be observed.

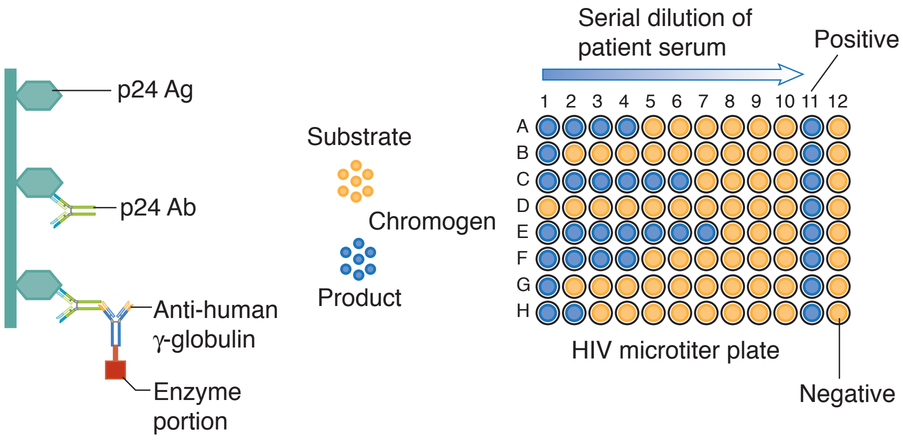


Figure I-9-15. ELISA Test

Fluorescence Activated Cell Sorting (FACS)

Fluorescence activated cell sorting (FACS) is a procedure used to rapidly analyze cell types in a complex mixture. This is done by sorting the cells into different populations based on their binding to specific fluorescently labelled antibodies. By using antibodies against cell-surface markers conjugated to different fluorescent dyes, it is possible to analyze the relative numbers of cells present in a specific tissue location.

As cells pass through the apparatus in a single file, a computer-generated graph is produced, plotting the intensity and color of fluorescence of each cell along the axes. Each dot on the graph reflects the passage of a cell with a certain level and color of fluorescence, so the darkly dotted areas of the graph reflect the presence of many cells of similar attributes.

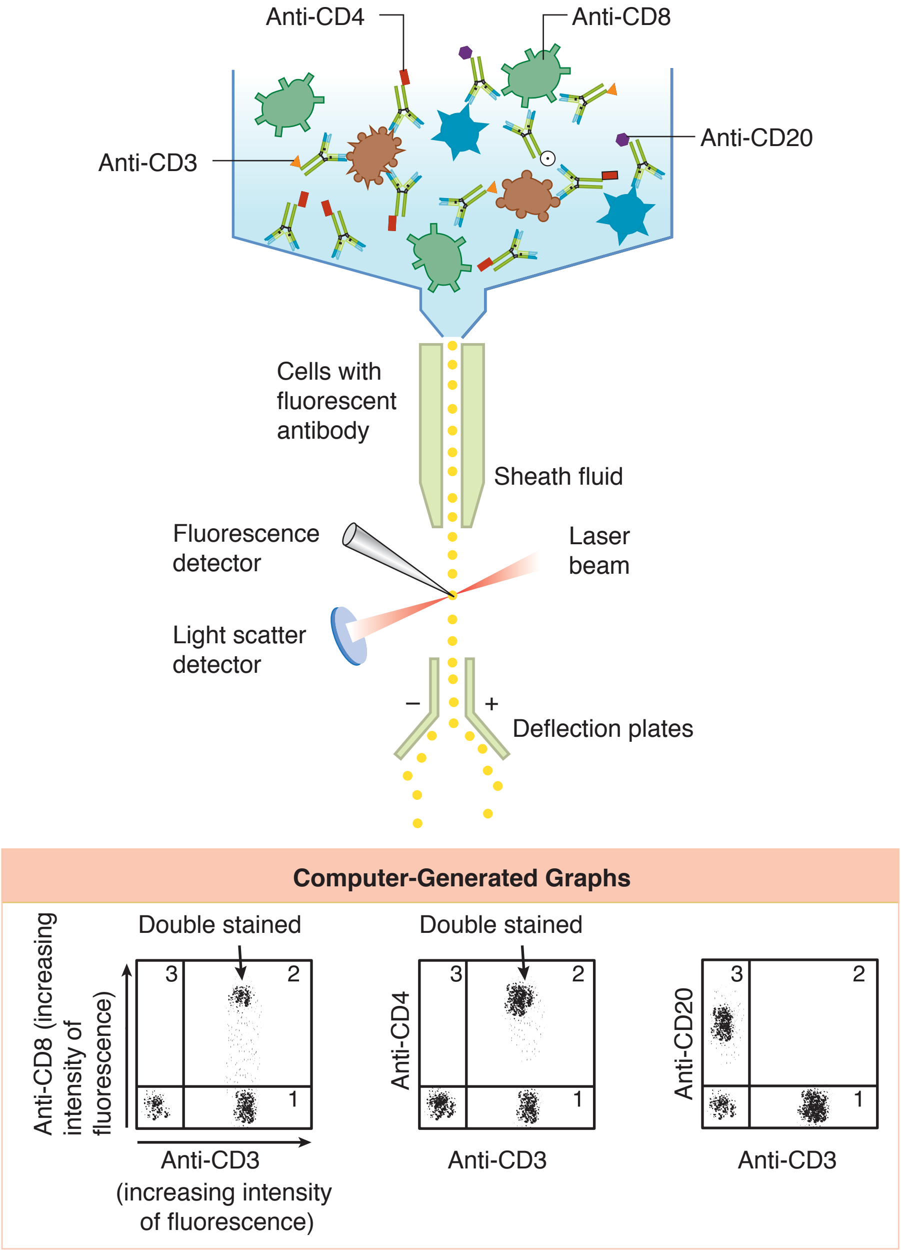


Figure I-9-16. Flow Cytometric Analysis

Immunizations 10

Learning Objectives

- ❏ Explain information related to vaccinations and secondary/subsequent responses
- ❏ Differentiate between killed, live, and component of vaccines
- ❏ Differentiate between bacterial and viral vaccines
- ❏ Answer questions about acquisition of immunoglobulins in the fetus and neonate
- ❏ Explain information related to childhood vaccine schedule

VACCINATION

Vaccination is a true milestone of medicine and has saved countless lives from preventable diseases. The concept dates back into the 1100s when the Chinese practiced the art of variolation. However, the practice is credited to Edward Jenner in 1798, when he used a strain of cowpox virus to protect a child from smallpox.

This chapter will discuss the science behind vaccination as well as a summary of the types of vaccine currently used in medicine.

SECONDARY AND SUBSEQUENT RESPONSES

When an antigen is introduced into the system a second time, the response of lymphocytes is accelerated and the result amplified over that of the primary immune response. The increased speed of this response is due to the presence of the memory-cell progeny of the primary response throughout the body. The increased amplitude of effector production is due to the fact that activation and cloning begin from a much larger pool of respondents.

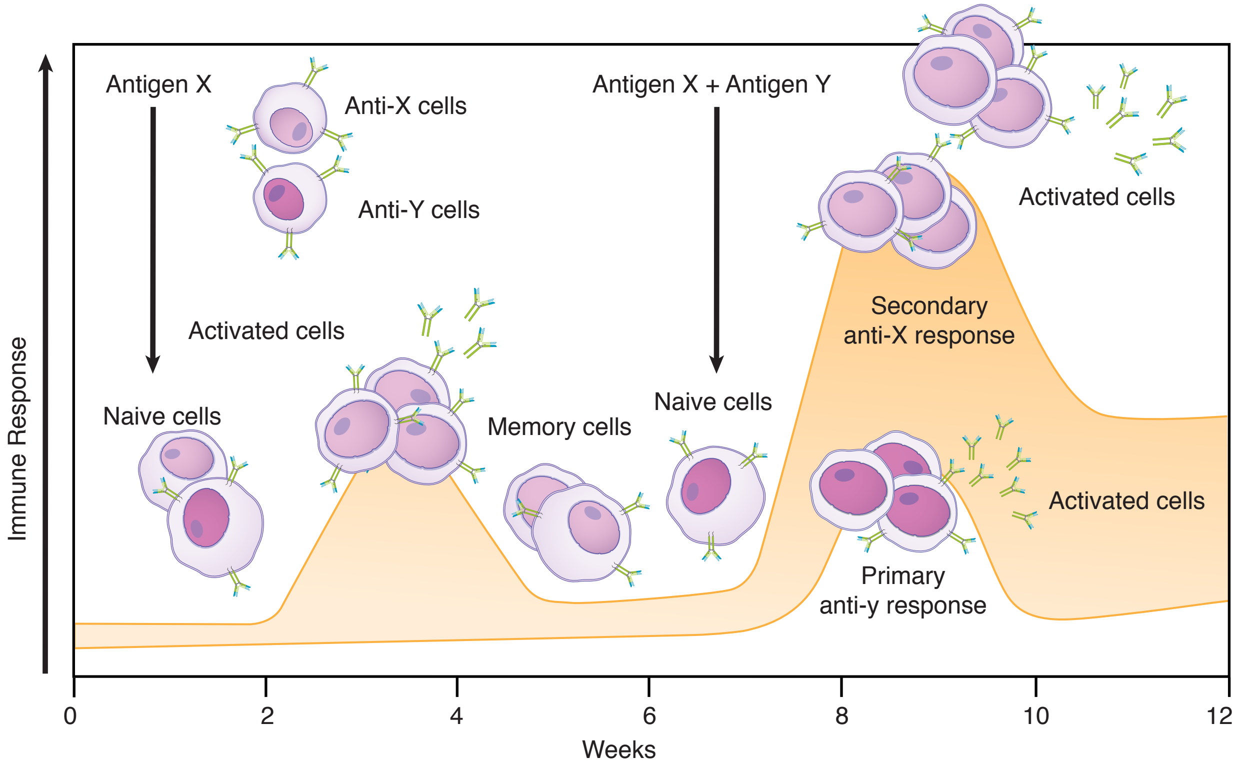


Figure I-10-1. Primary and Secondary Immune Responses

Table I-10-1. Primary versus Secondary Immune Response

Feature	Primary Response	Secondary Response
Time lag after immunization	5–10 days	1–3 days
Peak response	Small	Large
Antibody isotype	IgM, then IgG	Increasing IgG, IgA, or IgE
Antibody affinity	Variable to low	High (affinity maturation)
Inducing agent	All immunogens	**Protein** antigens
Immunization protocol	High dose of antigen (often with adjuvant)	Low dose of antigen (often without adjuvant)

TYPES OF IMMUNITY

Immunity to infectious organisms can be achieved by active or passive immunization. The goal of passive immunization is transient protection or alleviation of an existing condition, whereas the goal of active immunization is the elicitation of protective immunity and immunologic memory. Active and passive immunization can be achieved by both natural and artificial means.

Table I-10-2. Types of Immunity

Type of Immunity	Acquired Through	Examples
Natural	Passive means	Placental IgG transport, colostrum
Natural	Active means	Recovery from infection
Artificial	Passive means	Horse antivenin against black widow spider bite, snake bite Horse antitoxin against botulism, diphtheria Pooled human immune globulin versus hepatitis A and B, measles, rabies, varicella zoster or tetanus "Humanized" monoclonal antibodies versus RSV*
Artificial	Active means	Hepatitis B component vaccine Diphtheria, tetanus, pertussis toxoid vaccine *Haemophilus* capsular vaccine Polio live or inactivated vaccine Measles, mumps, rubella attenuated vaccine Varicella attenuated vaccine

*Monoclonal antibodies prepared in mice but spliced to the constant regions of human IgG

Passive Immunotherapy

Passive immunotherapy may be associated with several risks:

- Introduction of antibodies from other species can generate IgE antibodies, which may cause systemic anaphylaxis. The generation of IgE after infusion with even human gamma globulins is particularly an issue in persons with selective IgA deficiency (1:700 in population) as IgA is a molecule they have not encountered before. These patients can, however, be given IgA depleted globulins.
- Introduction of antibodies from other species can generate IgG or IgM anti-isotype antibodies, which form complement-activating immune complexes, leading to possible type III hypersensitivity reactions.
- Introduction of antibodies from humans can elicit responses against minor immunoglobulin polymorphisms or allotypes.

TYPES OF VACCINE

Live Vaccines

- **Attenuated** (attenuated = weak)
  - Comprised of **live** organisms which lose capacity to cause disease but still replicate in the host
  - Best at stimulating both a humoral and cell mediated immune response, as they mimic the natural infection and typically elicit lifelong immunity
  - Typically, 1 dose provides immunity but 2 doses are used to ensure seroconversion in most individuals
  - Dangerous for immunocompromised patients because even attenuated viruses can cause them significant disease; since attenuated vaccines are comprised of live organisms, there is slight potential to revert back to a virulent form
  - Live viral vaccines:
    - Recommended in the United States:
      - Measles, mumps and rubella (MMR)
      - Varicella zoster (VZV) (for both chicken pox and zoster [shingles])
      - Rotavirus
      - Influenza (flu-mist)
    - Available in the United States but recommended only under special circumstances:
      - Polio (Sabin)
      - Smallpox
      - Yellow fever
- **Non-attenuated**
  - Used by U.S. military against adenovirus types 4 and 7
  - Enteric coated, live, non-attenuated virus preparation
  - Produces an asymptomatic intestinal infection, thereby inducing mucosal IgA memory cells; these cells then populate the mucosal immune system throughout the body
  - Vaccine recipients are thus protected against adenovirus acquired by aerosol, which could otherwise produce pneumonia (this is the **only example** of a live non-attenuated vaccine that is used)

Killed Vaccines

- Utilize organisms that are killed so they can **no longer** replicate in the host
- Inactivated by chemicals rather than heat, as heat will often denature the immunogenic epitopes
- Typically require several doses to achieve desired response
- Predominantly produce humoral immunity

- Killed (inactivated) vaccines:
  - Rabies
  - Influenza
  - Polio (Salk)
  - Hepatitis A

Toxoid Vaccines

- Made from inactivated exotoxins from toxigenic bacteria
- Prevent disease but not infection
- Toxoid vaccines:
  - Diphtheria, tetanus, and acellular pertussis (DTaP)*

*The DTaP vaccine prep is composed of toxoids from both diphtheria and tetanus, while the pertussis comprises both filamentous hemagglutinin and inactivated pertussis toxin. The DTaP vaccine is considered safe with few side effects, and is the vaccine currently used in the United States.

Polysaccharide Vaccines

- Comprised of the capsular polysaccharide found in many bacteria
- Are only capable of producing IgM because of the inability of polysaccharide to activate Th cells (which require protein to become activated)
- Have largely been replaced with conjugate vaccines (see below)
- Polysaccharide vaccine(s):
  - *Streptococcus pneumoniae*, pneumococcal polysaccharide (PPSV23)
    - Comprised of 23 capsular serotypes of the most invasive and common strains of *S. pneumoniae*
    - Indicated for use in adults age >65 or special circumstances, i.e., splenectomy, COPD

Conjugate Vaccines

- Comprised of capsular polysaccharide conjugated to protein (usually a toxoid (see figure I-10-3); this creates a T cell-dependent immune response with class switching
- Creates a booster response to multiple doses
- Conjugate vaccines:
  - *Haemophilus influenzae type b* (Hib)
  - *Streptococcus pneumoniae*, Pneumococcal conjugate (PCV13)
    - Comprised of 13 capsular serotypes
    - Indicated for use in infants
  - *Neisseria meningitidis*

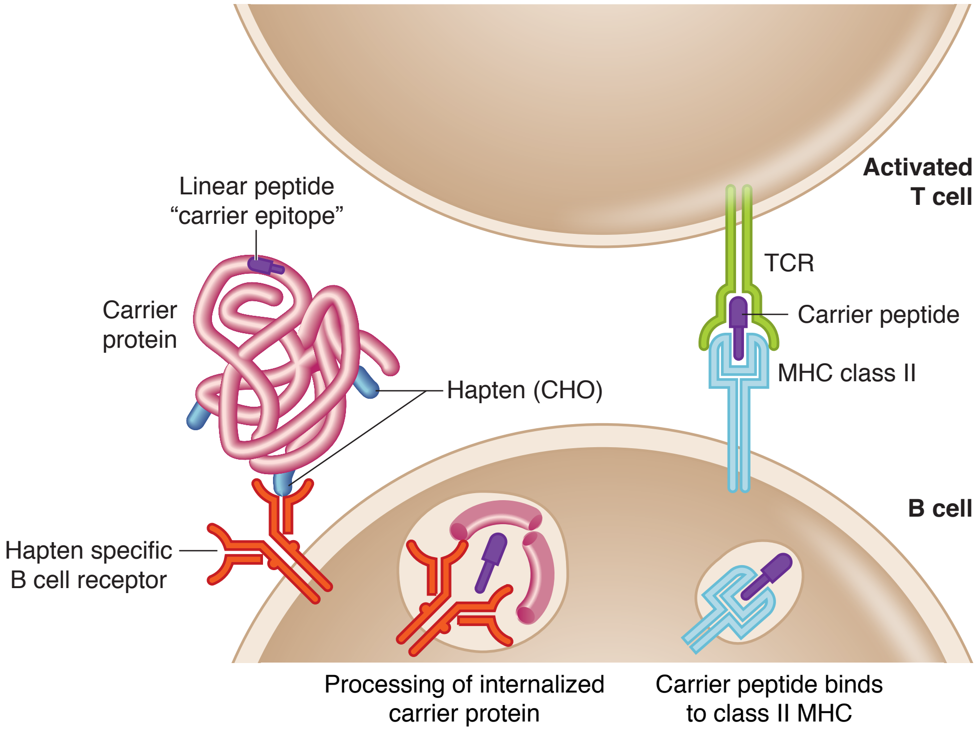


Figure I-10-2. Example of Conjugate Vaccine

Component Vaccines

- Comprised of an immunodominant protein from the virus that is grown in yeast cells
  - For example, in the hepatitis B vaccine, the gene coding for the HBsAg is inserted into yeast cells, which then releases this molecule into the culture medium; the molecule is then purified and used as the immunogen in the vaccine
- Component vaccines:
  - HBV
    - Hepatitis B surface antigen
  - HPV
    - Quadrivalent vaccine with serotypes 6, 11, 16 and 18
    - 9-valent vaccine (Gardasil 9) to prevent >90% of cancers, as opposed to the quadrivalent vaccine which can protect up to 70% of cancers; contains serotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58
      - Released in February 2015

ACQUISITION OF IMMUNOGLOBULINS IN THE FETUS AND NEONATE

Persistence of maternal Ab affects vaccinations.

- Live attenuated virus vaccines are given only age >12 months because residual maternal antibodies would inhibit replication and the vaccine would fail.
- When children are at exceptionally high risk for exposure to a pathogen, this rule is sometimes broken, but administration of vaccine at age <6–9 months is almost always associated with the need for repeated booster inoculations.
- IgM is the only isotype useful in diagnosing infections in neonates.
- Normal infants have few infections during first few months because of maternal IgG.
- Children with immune deficiencies don't become ill until maternal IgG is low.
- Infants have 20% of adult IgA at age 12 months, so colostrum is important.

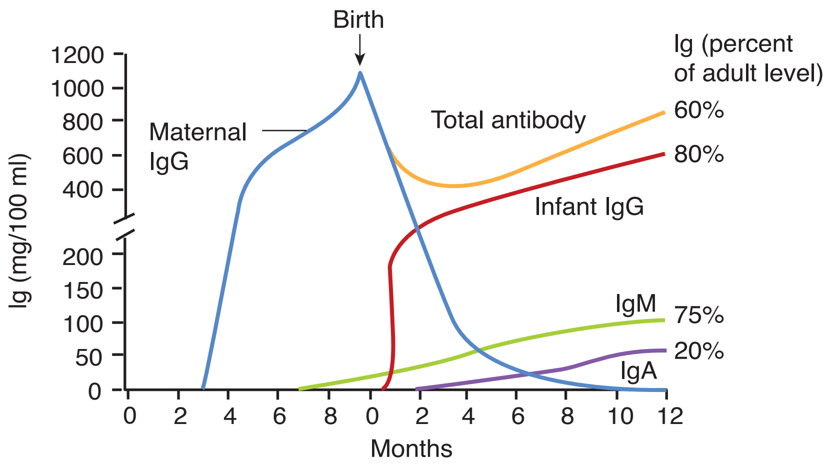


Figure I-10-3. Immunoglobulins in Serum of Fetus and Newborn Child

CHILDHOOD VACCINE SCHEDULE

The current recommended list of vaccines for children in the United States is listed below. The vaccines in this list are high yield.

Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger—United States, 2017.

(FOR THOSE WHO FALL BEHIND OR START LATE, SEE THE CATCH-UP SCHEDULE).

These recommendations must be read with the footnotes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars. To determine minimum intervals between doses, see the catch-up schedule. School entry and adolescent vaccine age groups are shaded in gray.

Vaccine	Birth	1 mo	2 mos	4 mos	6 mos	9 mos	12 mos	15 mos	18 mos	19-23 mos	2-3 yrs	4-6 yrs	7-10 yrs	11-12 yrs	13-15 yrs	16 yrs	17-18 yrs
Hepatitis B[1] (HepB)	1st dose	←2nd dose→					←3rd dose→										
Rotavirus[2] (RV) RV1 (2-dose series); RV5 (3-dose series)			1st dose	2nd dose	See footnote 2												
Diphtheria, tetanus, & acellular pertussis[3] (DTaP: <7 yrs)			1st dose	2nd dose	3rd dose			←4th dose→				5th dose					
Haemophilus influenzae type b[4] (Hib)			1st dose	2nd dose	See footnote 4		←3rd or 4th dose, See footnote 4→										
Pneumococcal conjugate[5] (PCV13)			1st dose	2nd dose	3rd dose		←4th dose→										
Inactivated poliovirus[6] (IPV: <18 yrs)			1st dose	2nd dose			←3rd dose→					4th dose					
Influenza[7] (IIV)									Annual vaccination (IIV) 1 or 2 doses						Annual vaccination (IIV) 1 dose only		
Measles, mumps, rubella[8] (MMR)					See footnote 8		←1st dose→					2nd dose					
Varicella[9] (VAR)							←1st dose→					2nd dose					
Hepatitis A[10] (HepA)								←2-dose series, See footnote 10→									
Meningococcal[11] (Hib-MenCY ≥6 weeks; MenACWY-D ≥9 mos; MenACWY-CRM ≥2 mos)						See footnote 11								1st dose		2nd dose	
Tetanus, diphtheria, & acellular pertussis[12] (Tdap: ≥7 yrs)														Tdap			
Human papillomavirus[13] (HPV)														See footnote 13			
Meningococcal B[11]															See footnote 11		
Pneumococcal polysaccharide[5] (PPSV23)														See footnote 5			

- Range of recommended ages for all children
- Range of recommended ages for catch-up immunization
- Range of recommended ages for certain high-risk groups
- Range of recommended ages for non-high-risk groups that may receive vaccine, subject to individual clinical decision making
- No recommendation

NOTE: The above recommendations must be read along with the footnotes of this schedule.

BACTERIAL VACCINES

Table I-10-3. Bacterial Vaccines

Organism	Vaccine	Vaccine Type
C. diphtheriae	**D**TaP	Toxoid
B. pertussis	DT**aP**	Toxoid plus filamentous hemagglutinin
C. tetani	D**T**aP	Toxoid
H. influenzae	Hib	Capsular polysaccharide and protein
S. pneumoniae	PCV Pediatric	13 capsular serotypes and protein
	PPV Adult	23 capsular serotypes
N. meningitidis	MCV-4	4 capsular serotypes (Y, W-135, C, A) and protein

VIRAL VACCINES

Table I-10-4. Viral Vaccines

Virus	Vaccine	Vaccine Type
Rotavirus	RV	Live
Polio	IPV	Killed (Salk)
	OPV	Live (Sabin)
Influenza	IIV	Inactivated (killed)
	LAIV	Live
Varicella zoster virus	VAR	Live
Hepatitis A	HepA	Inactivated (killed)
Human papilloma virus	HPV	Component
Hepatitis B virus	HepB	Component
Measles	MMR	Live
Mumps	MMR	Live
Rubella	MMR	Live

Primary Immunodeficiencies 11

Learning Objectives

- ❏ Solve problems concerning defects of phagocytic cells and humoral immunity
- ❏ Demonstrate understanding of deficiencies of complement or its regulation
- ❏ Use knowledge of defects of T lymphocytes to explain severe combined immunodeficiencies

Immunodeficiency diseases may occur with any aspect of immunity, including both the innate and adaptive branches. The symptoms of each disease highlight the importance of that aspect of the immune system on protecting the host. Most of these immune disorders are pediatric in nature and begin to appear around age 6 months. This highlights the importance of the protective immunity afforded by maternal IgG, which is nearly depleted by age 6 months and completely depleted by age 12-15 months.

Another important aspect of immunodeficiency diseases is that several are X-linked and therefore more common in males than females. Because these diseases reveal the importance of the immune system's basic function, they are often heavily tested.

DEFECTS OF PHAGOCYTIC CELLS

Table I-11-1. Defects of Phagocytic Cells

Disease	Molecular Defect(s)	Symptoms
Chronic granulomatous disease (CGD)	Deficiency of **NADPH oxidase** (any one of 4 component proteins); failure to generate superoxide anion, other O_2 radicals	Recurrent infections with **catalase-positive** bacteria and fungi
Leukocyte adhesion deficiency	Absence of **CD18**—common β chain of the leukocyte integrins The 3 integrins that contain CD18: LFA-1, MAC-1 and gp150/95	Recurrent and chronic infections, failure to form pus, and delayed separation of umbilical cord stump
Chediak-Higashi syndrome	Nonsense mutation in the lysosomal trafficking regulator, CHS1/LYST protein, leads to aberrant fusion of vesicles	Recurrent infection with bacteria: chemotactic and degranulation defects; absent NK activity, partial **albinism**
Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Deficiency of essential enzyme in hexose monophosphate shunt	Same as CGD, with associated **anemia**
Myeloperoxidase deficiency	Defect in MPO affects the ability to convert hydrogen peroxide to hypochlorite	Mild or none
Hyperimmunoglobulin E syndrome (formerly Job syndrome)	Defects in JAK-STAT signaling pathway leading to impaired Th17 function: decreased IFN-gamma production	Characteristic facies, severe, recurrent sinopulmonary infections, pathologic bone fractures, retention of primary teeth, increased IgE, eczematous rash

DEFECTS OF HUMORAL IMMUNITY

Table I-11-2. Defects of Humoral Immunity

Disease	Molecular Defect	Symptoms/Signs	Treatment
Bruton (X-linked) agammaglobulinemia	Deficiency of the Bruton tyrosine kinase (btk) which promotes pre-B cell expansion; faulty B-cell development	Increased susceptibility to encapsulated bacteria and bloodborne viruses, low immunoglobulins of all isotypes, absent or low levels of circulating B-cells. B-cell maturation does not progress past the pre-B cell stage while maintaining cell-mediated immunity.	Monthly gamma-globulin replacement, antibiotics for infection
X-linked hyper-IgM syndrome	Deficiency of **CD40L** on activated T cells	**High serum titers of IgM without other isotypes,** normal B and T-cell numbers, susceptibility to encapsulated bacteria and opportunistic pathogens.	Antibiotics and gammaglobulins
Selective IgA deficiency	Multiple genetic causes	Decreased IgA levels and normal IgM and IgG with elevation of IgE. Repeated **sinopulmonary and gastrointestinal** infections, ↑ **atopy**	Antibiotics, not immunoglobulins
Common variable Immunodeficiency	Collection of syndromes; several associated genetic defects	Onsets in **late teens**, early twenties; B cells present in peripheral blood, immunoglobulin levels decrease with time; ↑ autoimmunity	Antibiotics
Transient hypogammaglob-ulinemia of infancy	Delayed onset of normal IgG synthesis	Detected in month 5–6 of life, resolves months 16–30; susceptibility to pyogenic bacteria	Antibiotics and in severe cases, gamma-globulin replacement

DEFICIENCIES OF COMPLEMENT OR ITS REGULATION

Table I-11-3. Deficiencies of Complement or Its Regulation

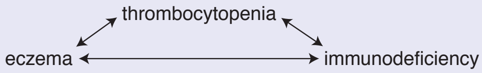
Deficiencies in Complement Components	Deficiency	Signs/Diagnosis
Classic pathway	C1q, C1r, C1s, C4, C2	Marked increase in immune complex diseases, increased infections with pyogenic bacteria
Both pathways	C3	Recurrent bacterial infections, immune complex disease
	C5, C6, C7, C8, or C9	Recurrent **meningococcal and gonococcal infections**
Deficiencies in complement regulatory proteins	C1-INH **(hereditary angioedema)**	Overuse of C1, C4, or C2, edema at mucosal surfaces

DEFECTS OF T LYMPHOCYTES AND SEVERE COMBINED IMMUNODEFICIENCIES

Although patients with defects in B lymphocytes can deal with many pathogens adequately, defects in T lymphocytes are observed globally throughout the immune system. Because of the central role of T cells in activation, proliferation, differentiation, and modulation of virtually all naturally occurring immune responses, abnormalities in these cell lines send shock waves throughout the system. It is often a Herculean clinical effort to dissect the cause-and-effect relationships in such inherited diseases, and their diagnosis is often one of trial-and-error, which takes years to unravel.

Although in some cases both B- and T-lymphocyte defects may occur, the initial manifestation of these diseases is almost always infection with agents such as **fungi and viruses** that are normally destroyed by T-cell–mediated immunity. The B-cell defect, if any, is usually not detected for the first few months of life because of the passive transfer of immunoglobulins from the mother through the placenta or colostrum. The immune system is so compromised that even attenuated vaccine preparations can cause infection and disease.

Table I-11-4. T-Cell Deficiencies and Combined Deficiencies

Category	Disease	Defect	Clinical Manifestations
Selective T-cell deficiency	**DiGeorge Syndrome**	Heterozygous deletion of chromosome 22q11; failure of formation of **3rd and 4th pharyngeal pouches**, thymic aplasia	Characteristic facies and a clinical triad of cardiac malformations, hypocalcemia and hypoplastic thymus
	MHC class I deficiency	Failure of TAP 1 molecules to transport peptides to endoplasmic reticulum	**CD8+ T cells deficient, CD4+ T cells normal**, recurring viral infections, normal DTH, normal Ab production
Combined partial B- and T-cell deficiency	**Wiskott-Aldrich Syndrome**	Defect in the WAS protein which plays a critical role in actin cytoskeleton rearrangement	Defective responses to bacterial polysaccharides and depressed IgM, gradual **loss of humoral and cellular responses, thrombocytopenia, and eczema** IgA and IgE may be elevated 
	Ataxia telangiectasia	Defect in the ATM kinase involved in the detection of DNA damage and progression through the cell cycle	**Ataxia** (gait abnormalities), **telangiectasia** (capillary distortions in the eye), **deficiency of IgA and IgE** production
Complete functional B- and T-cell deficiency	Severe combined immunodeficiency (SCID)	Defects in common γ chain of IL-2 receptor (present in receptors for IL-4, −7, −9, −15), X-linked	Chronic diarrhea; skin, mouth, and throat lesions; opportunistic (**fungal**) infections; low levels of circulating lymphocytes; cells unresponsive to mitogens
		Adenosine deaminase deficiency (results in toxic metabolic products in cells)	Clinical overlap with X-linked SCID plus neurologic deficiency
		*rag*1 or *rag*2 gene nonsense mutations	Total absence B+ T cells
	Bare lymphocyte syndrome/**MHC class II deficiency**	Failure of MHC class II expression, defects in transcription factors	T cells present and responsive to nonspecific mitogens, no GVHD, **deficient in CD4+ T cells**, hypogammaglobulinemia. Clinically observed as a severe combined immunodeficiency

Hypersensitivity and Autoimmune Disease 12

Learning Objectives

- ❑ Differentiate type I (immediate), type II (antibody-mediated), type III (immune complex), and type IV (T-cell-mediated) hypersensitivity
- ❑ Answer questions about the pathogenesis of autoimmunity

Hypersensitivity diseases are conditions in which tissue damage is caused by immune responses. They may result from uncontrolled or excessive responses against **foreign** antigens or from a **failure of self-tolerance**, in which case they are called **autoimmune diseases**.

The 2 principal factors which determine the clinical and pathologic consequences of such conditions are the **type of immune response** elicited and the **nature and location of the inciting antigen**.

What the hypersensitivity reactions have in common:

- The first exposure to the antigen "sensitizes" lymphocytes.
- Subsequent exposures elicit a damaging reaction.
- The response is specific to a particular antigen or a cross-reacting substance.

Hypersensitivity diseases are classified on the basis of the effector mechanism responsible for tissue injury, and 4 types are commonly recognized.

Table I-12-1. Classification of Immunologic Diseases

Type of Hypersensitivity	Immune Mechanisms	Mechanisms of Tissue Injury
Immediate (type I)	Activation of Th2 cells resulting in the production of IgE which in turn binds to FcεR on mast cells, basophils and eosinophils	**Immediate reaction** • Degranulation and release of vasoactive amines (ie. histamine) and proteases **Late-phase reaction** • Synthesis and secretion of prostaglandins and leukotrienes • Cytokine-induced inflammation and leukocyte recruitment
Antibody-mediated (type II)	IgM and IgG against surface (cell surface or extracellular matrix)	**Complement-mediated (cytotoxic)** • Opsonization and enhances phagocytosis • Recruitment and activation of inflammatory cells **Non-cytotoxic** • Change in physiologic behavior of a cell
Immune complex–mediated (type III)	Deposition of immune complexes comprised of IgM or IgG and soluble antigen	Complement-mediated recruitment and activation of inflammatory cells resulting in some combination of arthritis, vasculitis and/or nephritis.
Delayed-type hypersensitivity (type IV)	Inflammatory cytokines, IFN-γ and IL-17, produced by CD4+ Th1 and Th17 cells, respectively.	**Cytokine-mediated tissue damage** • IFN-γ activation of macrophage • IL-17 recruitment and activation of neutrophil **Direct killing** • CTL-mediated cellular death
		CD8+ CTLs (T-cell-mediated cytolysis) Direct target cell killing, cytokine-mediated inflammation

TYPE I (IMMEDIATE) HYPERSENSITIVITY

Type I is the only type of hypersensitivity mediated by IgE antibodies and mast cells. It is manifested within minutes of the reexposure to an antigen. The IgE response is the normal protective response against many metazoan parasites, which are too large to be phagocytized or killed by other cytopathic mechanisms. Approximately 20% of all individuals in the United States, however, display this immune response against harmless environmental antigens, such as pet dander or pollen; these responses are called **atopic** or **allergic** responses.

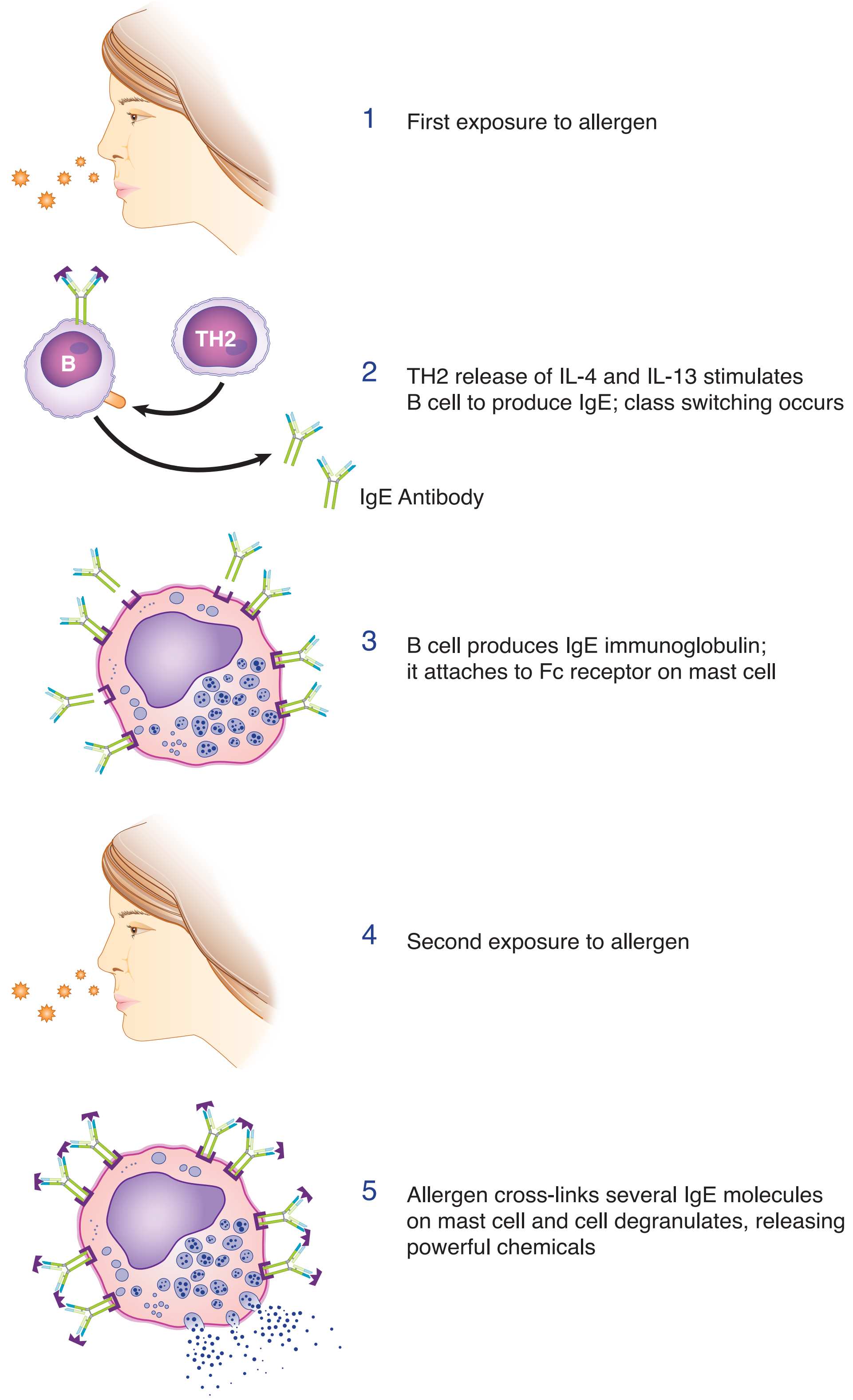


Figure I-12-1. Development of the Immediate Hypersensitivity Reaction

The effector cells of the immediate hypersensitivity reaction are mast cells, basophils, and eosinophils. The soluble substances they release into the site cause the symptoms of the reaction. Approximately 2-4 hours after the immediate response to release of these mediators, a **late-phase reaction** is mediated by products of the arachidonic acid cascade.

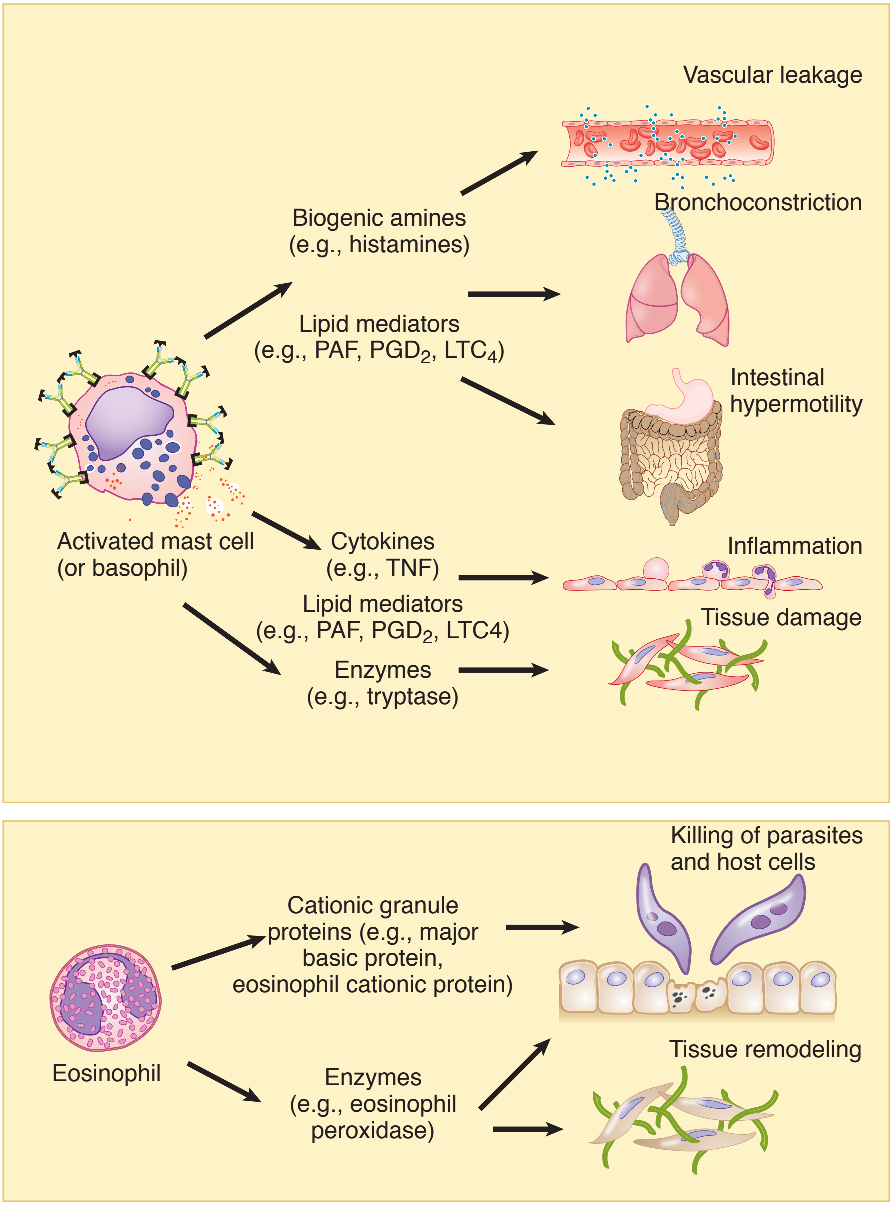


Figure I-12-2. Mediators of Type I Hypersensitivity

Table I-12-2. Mast Cell Mediators

Mediators Stored and Released	Effect
Histamine	Smooth muscle contraction; increased vascular permeability
Heparin	Anticoagulant
Eosinophil chemotactic factor A (multiple chemokines)	Chemotactic
Mediators Newly Synthesized from Arachidonic Acid	**Effect**
Prostaglandin D_2, E_2, $F_{2\alpha}$	Increased smooth muscle contraction and vascular permeability
Leukotrienes C_4, D_4, E_4 (lipoxygenase pathway)	Increased smooth muscle contraction and vascular permeability
Leukotriene B_4	Chemotactic for neutrophils

Table I-12-3. Allergic Diseases Due to Specific Allergens and Their Clinical Manifestations

Allergic Disease	Allergens	Clinical Findings
Allergic rhinitis (hay fever)	Trees, grasses, dust, cats, dogs, mites	Edema, irritation, mucus in nasal mucosa
Systemic anaphylaxis	**Insect stings, drug reactions**	Bronchial and tracheal constriction, complete vasodilation and death
Food allergies	Milk, eggs, fish, cereals, grains	Hives and gastrointestinal problems
Wheal and flare	In vivo skin testing for allergies	Local skin edema, reddening, vasodilation of vessels
Asthma	Inhaled materials	Bronchial and tracheal constriction, edema, mucus production, massive inflammation

TYPE II (ANTIBODY-MEDIATED) HYPERSENSITIVITY

Antibodies against cell surface or extracellular matrix antigens cause diseases that are **specific to the tissues** where those antigens are present; they are not usually systemic. In most cases, these antibodies are **autoantibodies**, but they may be produced against a foreign antigen that is cross-reactive with self-components of tissues.

These antibodies can cause tissue damage by 3 main mechanisms:

- Opsonization of cells
- Activation of the complement system which recruit neutrophils and macrophages that cause tissue damage
- Possible binding to normal cellular receptors and interference with their function

In some types of type II hypersensitivity, complement is activated and/or ADCC is active (e.g., hemolytic disease of the newborn [HDNB]). In other types, cell function is altered in the absence of complement activation and ADCC (e.g., myasthenia gravis and Graves disease). Eventually, as these diseases progress, complexes of antigen and antibody may cause localized damage, but they **do not circulate** so the damage is localized to the specific tissue.

Table I-12-4. Type II Hypersensitivities

Disease	Target Antigen	Mechanism of Pathogenesis	Clinical Manifestations
Cytotoxic			
Autoimmune hemolytic anemia (HDNB)	RBC membrane proteins (Rh, I Ags)	Opsonization, phagocytosis, and complement-mediated destruction of RBCs	Hemolysis, anemia
Acute rheumatic fever	**Streptococcal cell-wall Ag;** Ab cross-reacts with myocardial Ag	Inflammation, macrophage activation	**Myocarditis, arthritis**
Goodpasture syndrome	**Type IV collagen** in basement membranes of kidney glomeruli and lung alveoli	Complement- and Fc-receptor–mediated inflammation	**Nephritis,** lung hemorrhage, **linear Ab deposits**
Transfusion reaction	ABO blood glycoproteins	IgM isohemagglutinins formed naturally in response to normal bacterial flora cause opsonization + complement activation	Hemolysis
Autoimmune thrombocytopenic purpura	Platelet membrane proteins	Ab-mediated platelet destruction through opsonization and complement activation	Bleeding
Non-cytotoxic			
Myasthenia gravis	**Acetylcholine receptor**	Ab inhibits acetylcholine binding, downmodulates receptors	**Muscle weakness, paralysis**
Graves disease	**TSH receptor**	Ab-mediated stimulation of TSH receptors	**Hyperthyroidism followed by hypothyroidism**
Type II (insulin-resistant) diabetes	Insulin receptor	Ab inhibits binding of insulin	Hyperglycemia
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor, decreased absorption of vitamin B12	Abnormal erythropoiesis, anemia

An important example of type II hypersensitivity is **HDNB**, also known as erythroblastosis fetalis. In the fetus, this disease is due to transport of IgG specific for one of the Rhesus (Rh) protein antigens (RhD) across the placenta.

About 85% of people are Rh+. If a pregnant woman is Rh– and the father is Rh+, there is a chance that the fetus will also be Rh+. This situation will pose no problem in the first pregnancy, as the mother's immune system will not usually encounter fetal blood cell antigens until placental separation at the time of birth. At that time, however, Rh+ fetal red blood cells will enter the maternal circulation and stimulate a T-dependent immune response, eventually resulting in the generation of memory B cells capable of producing IgG antibody against RhD.

In a subsequent pregnancy with another Rh+ fetus, this maternal IgG can be transported across the placenta, react with fetal Rh+ red cells, and activate complement, producing hemolytic disease. Hemolytic disease of the newborn can be prevented by treating the Rh– mother with RhoGAM™, a preparation of human anti-RhD antibody, at 28 weeks of gestation and again within 72 hours after birth. This antibody effectively eliminates the fetal Rh+ cells before they can generate RhD-specific memory B cells in the mother. Anti-RhD antibody should be given to any Rh– individual following any termination of pregnancy.

TYPE III (IMMUNE COMPLEX) HYPERSENSITIVITY

The immune complexes that cause disease may involve either self or foreign antigens bound to antibodies. These immune complexes are filtered out of the circulation in the small vasculature, so their sites of ultimate damage do not reflect their sites of origin. These diseases tend to be systemic, with little tissue or organ specificity.

Table I-12-5. Type III Hypersensitivities

Disease	Antigen Involved	Clinical Manifestations
Systemic lupus erythematosus+	**dsDNA,** Sm, other nucleoproteins	Nephritis, arthritis, vasculitis, **butterfly facial rash**
Poststreptococcal glomerulonephritis	Streptococcal cell wall Ags (may be "planted" in glomerular basement membrane)	Nephritis, **"lumpy-bumpy" deposits**
Arthus reaction	Any injected protein	Local pain and edema
Serum sickness	Various proteins	Arthritis, vasculitis, nephritis
Polyarteritis nodosa	Hepatitis B virus Ag	Systemic vasculitis

+Other autoimmune diseases correlated with production of antinuclear antibodies include diffuse systemic sclerosis (antibodies to DNA topoisomerase 1), limited scleroderma (CREST; antibodies to centromeric proteins) and Sjögren syndrome (antibodies to ribonucleoproteins).

TYPE IV (T-CELL–MEDIATED) HYPERSENSITIVITY

T lymphocytes may cause tissue injury by triggering delayed-type hypersensitivity (DTH) reactions or by directly killing target cells. These reactions are elicited by CD4+ Th1, Th17 cells, or CD8+ CTLs, which activate macrophages, recruit neutrophils, and induce inflammation. These T cells may be autoreactive or specific

against foreign protein antigens bound to tissues. T-cell-mediated tissue injury is common during the protective immune response against persistent intracellular microbes.

Table I-12-6. Type IV Hypersensitivities

Disease	Specificity of Pathogenic T Cells	Clinical Manifestations
Tuberculin test	PPD (tuberculin & mycolic acid)	Indurated skin lesion (granuloma)
Contact dermatitis	**Nickel, poison ivy/oak catechols, hapten/carrier**	Vesicular skin lesions, pruritus, rash
Hashimoto thyroiditis*	Unknown Ag in thyroid	**Hypothyroidism**
Multiple sclerosis	Myelin Basic Protein	Progressive demyelination, blurred vision, paralysis
Rheumatoid arthritis*	Unknown Ag in joint synovium (type II collagen?)	Rheumatoid factor (IgM against Fc region of IgG), alpha-cyclic citrullinated peptide (α-CCP) antibodies, chronic arthritis, inflammation, destruction of articular cartilage and bone
Insulin-dependent diabetes mellitus (type I)*	Islet-cell antigens, insulin, glutamic acid decarboxylase, others	Chronic inflammation and destruction of β cells, polydipsia, polyuria, polyphagia, ketoacidosis
Guillain-Barré syndrome*	Peripheral nerve myelin or gangliosides	Ascending paralysis, peripheral nerve demyelination
Celiac disease	CD4+ cells—gliadin, CD8+ cells—HLA class I-like molecule expressed during stress	Gluten-sensitive enteropathy
Crohn disease	Unknown Ag, commensal bacteria?	Chronic intestinal inflammation due to Th1 and Th17 cells, obstruction

*Diseases classified at type IV pathologies in which autoantibodies are present and used as clinical markers

THE PATHOGENESIS OF AUTOIMMUNITY

The key factor in the development of autoimmunity is the recognition of self-antigens by autoreactive lymphocytes, which then become activated, proliferate, and differentiate to produce effector cells and cytokines that cause tissue injury. Autoimmunity must initially result from a failure of mechanisms of **central tolerance,** as cells are "educated" in the bone marrow and thymus (*see* chapter 3).

Self-reactive lymphocytes that escaped central tolerance are subject to the different mechanisms of peripheral tolerance. The 3 primary mechanisms that induce peripheral tolerance are anergy, deletion and suppression.

B lymphocytes that recognize self-antigen in the absence of the T-cell signaling become anergic and express high levels of IgD on their surface, excluding them from secondary lymphoid tissues. Anergic B lymphocytes are then unable to receive the signals necessary for survival and undergo apoptosis. Additionally, B lymphocytes have inhibitory receptors that can be engaged when self-antigen is recognized suppressing their activity.

Similar to self-reactive B lymphocytes, T lymphocytes that recognize self-antigen in the absence of the appropriate costimulatory signals are subject to anergy or deletion. Anergy is the result of a breakdown in either TCR signaling or the binding of an inhibitory receptor, CTLA-4 or PD-1. Deletion of self-reactive T lymphocytes is due to apoptosis by activation of the caspase signaling pathway or the Fas signaling pathway.

Self-reactive T lymphocytes are also subject to suppression by Tregs. Although a majority of Tregs are generated during central tolerance, some arise in the periphery. Tregs secrete IL-10 and TGF-beta that inhibit the activation of lymphocytes, macrophage and dendritic cells. CTLA-4 is expressed at high levels on Tregs and is thought to bind to and sequester the costimulatory molecule B7 which would otherwise be used to activate T lymphocytes.

Development of autoimmune disease is due to a combination of genetic and environmental factors as well as hormonal triggers. Among the strongest genetic associations with the development of autoimmune disease are the **HLA genes**. Also known to contribute to autoimmunity are polymorphisms in non-HLA genes.

Table I-12-7. Examples of HLA-Linked Immunologic Diseases

Disease	HLA Allele
Rheumatoid arthritis	DR4
Insulin-dependent diabetes mellitus	DR3/DR4
Multiple sclerosis, Goodpasture’s	DR2
Systemic lupus erythematosus	DR2/DR3
Ankylosing spondylitis, psoriasis, inflammatory bowel disease, reactive arthritis	B27
Celiac disease	DQ2 or DQ8
Graves disease	B8

Infections and tissue injury may alter the way that self-antigens are presented to lymphocytes and serve as an inciting factor in the development of disease. Because autoimmune reactions against one self-antigen may injure other tissues and expose other potential self-antigens for recognition, autoimmune diseases tend to be chronic and progressive.

Transplantation 13

Learning Objectives

- ❑ Solve problems concerning definitions
- ❑ Use knowledge of mechanisms of graft rejection
- ❑ Answer questions about graft versus host disease

OVERVIEW

Transplantation is the process of taking cells, tissues, or organs (a **graft**) from one individual (the **donor**) and implanting them into another individual or another site in the same individual (the **host** or **recipient**). **Transfusion** is a special case of transplantation and the most frequently practiced today, in which circulating blood cells or plasma are infused from one individual into another. As we have seen in previous chapters, the immune system is elaborately evolved to recognize minor differences in self antigens that reflect the invasion of harmful microbes or pathologic processes, such as cancer. Unfortunately, it is this same powerful mechanism of self-protection which thwarts tissue transplantation because tissues derived from other individuals are recognized as **"altered-self"** by the educated cells of the host's immune system.

Types of Graft Tissue

Several different types of grafts are used in medicine:

- Autologous grafts (or **autografts**) are those where tissue is moved from one location to another in the same individual (skin grafting in burns or coronary artery replacement with saphenous veins).
- **Isografts (or syngeneic grafts)** are those transplanted between genetically identical individuals (monozygotic twins).
- **Allogeneic** grafts are those transplanted between genetically different members of the same species (kidney transplant).
- **Xenogeneic** grafts are those transplanted between members of different species (pig heart valves into human).

MECHANISMS OF GRAFT REJECTION

The recognition of transplanted cells as self or foreign is determined by the extremely polymorphic genes of the major histocompatibility complex, which are expressed in a **codominant** fashion. This means that each individual inherits a complete set or **haplotype** from each parent and virtually assures that 2 genetically unrelated individuals will have distinctive differences in the antigens expressed on their cells.

Note

MHC alleles are expressed codominantly.

The net result is that all grafts except autografts are ultimately identified as foreign invading proteins and destroyed by the process of **graft rejection**. Even syngeneic grafts between identical twins can express recognizable antigenic differences due to somatic mutations that occur during the development of the individual. For this reason, all grafts except autografts must be followed by some degree of lifelong immunosuppression of the host to attempt to avoid rejection reactions.

The time sequence of allograft rejection differs depending on the tissue involved but always displays specificity and memory. As the graft becomes vascularized, CD4+ and CD8+ cells that migrate into the graft from the host become sensitized and proliferate in response to both major and minor histocompatibility differences. In the **effector phase** of the rejection, Th cytokines play a critical role in stimulating macrophage, cytotoxic T cell, and even antibody-mediated killing. Interferons and TNF-α and -β all increase the expression of class I MHC molecules in the graft, and IFN-γ increases the expression of class II MHC as well, increasing the susceptibility of cells in the graft to MHC-restricted killing.

Allograft rejection phenomena are classified according to their time of activation and the type of effector mechanism that predominates.

Hyperacute Graft Rejection

- Occurs within minutes to hours
- Due to pre-formed antibodies due to transfusions, multi-parity, or previous organ transplants (type II cytotoxic hypersensitivity)
- Antibodies bind to the grafted tissue and activate complement and the clotting cascade resulting in thrombosis and ischemic necrosis
- Rare because of cross-matching blood, **but common vignette**

Acute Graft Rejection

- Occurs within days to weeks; the timing and mechanism are similar to a primary immune response
- Induced by alloantigens (predominantly MHC) in the graft
- Both CD4 and CD8 T cells play a role as well as antibodies (think normal immune response)
- Immunosuppressive therapy works to prevent this type of graft rejection mainly

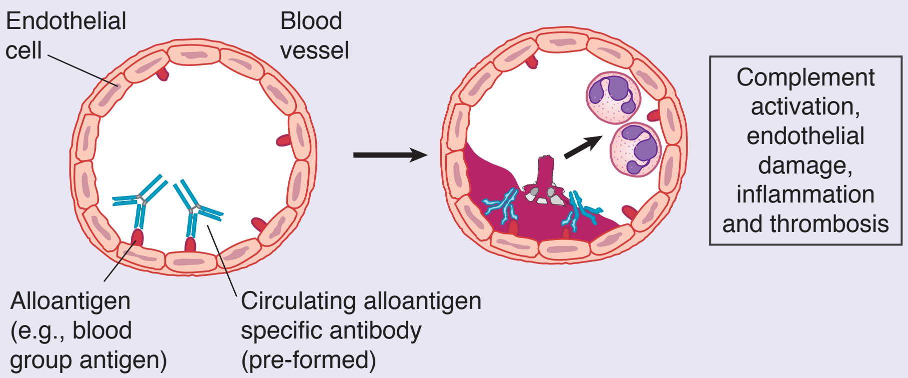
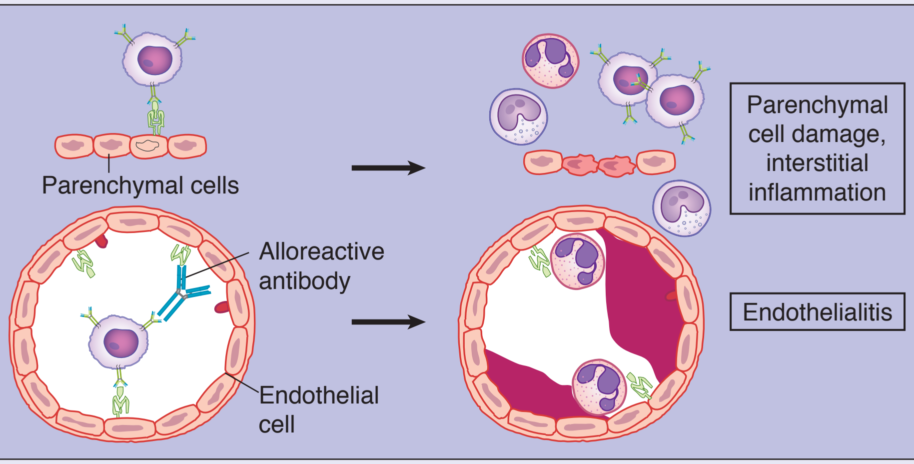
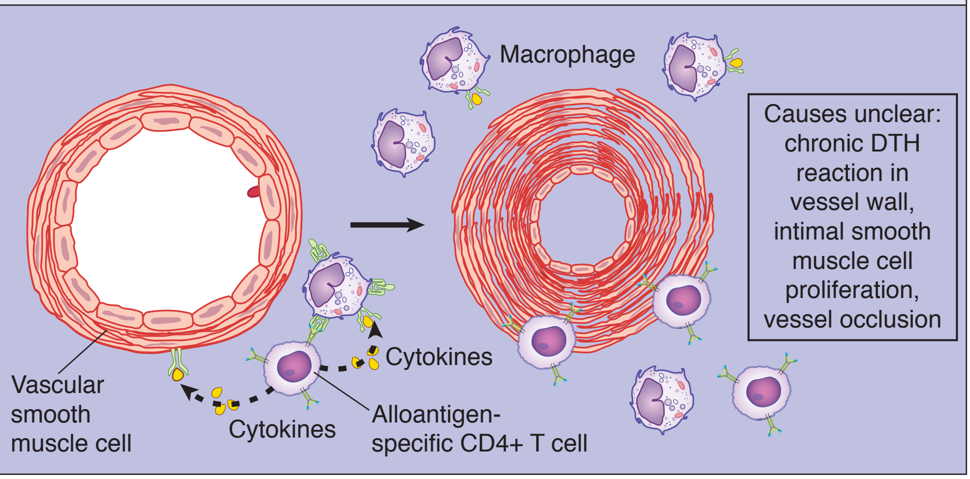
Accelerated Acute Graft Rejection

- Occurs within days; the timing and mechanism are similar to a memory response.

Chronic Graft Rejection

- Occurs within months to years
- Predominantly T cell mediated
- Difficult to treat and usually results in graft rejection
- Etiology not well understood, possibly triggered by viral infections

Table I-13-1. Type and Tempo of Rejection Reactions

Type of Rejection	Time Taken	Mechanism & Pathogenesis
Hyperacute rejection	Minutes to hours	
Acute rejection	Days to weeks	
Accelerated acute rejection	Days	As above, but mediated by memory cell responses
Chronic rejection	Months to years	

GRAFT VERSUS HOST DISEASE

A special case of tissue transplantation occurs when the grafted tissue is bone marrow. Because the bone marrow is the source of pluripotent hematopoietic stem cells, it can be used to reconstitute myeloid, erythroid, and lymphoid cells in a recipient who has lost these cells as a result of malignancy or chemotherapeutic regimens. Because the bone marrow is a source of some mature T lymphocytes, it is necessary to remove these cells before transplantation to avoid the appearance of **graft-versus-host disease** in the recipient. In this special case of rejection, any mature T cells remaining in the bone marrow inoculum can attack allogeneic MHC-bearing cells of the recipient and cause widespread epithelial cell death accompanied by rash, jaundice, diarrhea, and gastrointestinal hemorrhage.

Clinical Correlate

Monoclonal antibodies are used in the treatment and prevention of graft rejection along with the classic therapies (corticosteroids, cyclosporine A, rapamycin, etc.).

Drug/Target	Mechanism of Action
Daclizumab, basiliximab (anti-IL-2 receptor antibody)	Blocks T cell proliferation via blocking the binding of IL-2, opsonization of IL-2R bearing cells
Muromonab (anti-CD3)	Blocks T cell activation by causing apoptosis
Belatacept (CTLA-4-Ig)	Inhibits T cell activation by blocking the B7 costimulatory molecule binding to CD28
Alemtuzumab (anti-CD52)*	Depletes pool of T cells by binding to them and causing complement mediated lysis

*CD52 is a marker found on all lymphocytes.

APPENDIX I

CD Markers

CD Designation	Cellular Expression	Known Functions
CD2 (LFA-2)	T cells, thymocytes, NK cells	Adhesion molecule
CD3	T cells, thymocytes	Signal transduction by the TCR
CD4	Th cells, thymocytes, monocytes, and macrophages	Coreceptor for TCR-MHC II interaction, receptor for HIV
CD8	CTLs, some thymocytes	Coreceptor for MHC class I–restricted T cells
CD14 (LPS receptor)	Monocytes, macrophages, granulocytes	Binds LPS
CD16 (Fc receptor)	NK cells, macrophages, neutrophils	Opsonization ADCC
CD18	Leukocytes	Cell adhesion molecule (missing in leukocyte adhesion deficiency)
CD19	B cells	Coreceptor with CD21 for B-cell activation (signal transduction)
CD20	Most or all B cells	Unknown role in B-cell activation
CD21 (CR2, C3d receptor)	Mature B cells	Receptor for complement fragment C3d, forms coreceptor complex with CD19, Epstein-Barr virus receptor
CD25	Activated Th cells and T_{Reg}	Alpha chain of IL-2 receptor
CD28	T cells	T-cell receptor for costimulatory molecule B7
CD34	Precursors of hematopoietic cells, endothelial cells in HEV	Cell–cell adhesion, binds L-selectin
CD40	B cells, macrophages, dendritic cells, endothelial cells	Binds CD40L, role in T-cell–dependent B cell, macrophage, dendritic cell and endothelial cell activation
CD56	NK cells	Cell adhesion
CD152 (CTLA-4)	Activated T cells	Negative regulation: competes with CD28 for B7 binding

APPENDIX II

Cytokines

Cytokine	Secreted by	Target Cell/Tissue	Activity
Interleukin (IL)-1	Monocytes, macrophages, B cells, dendritic cells, endothelial cells, others	Th cells	Costimulates activation
		B cells	Promotes maturation and clonal expansion
		NK cells	Enhances activity
		Endothelial cells	Increases expression of ICAMs
		Macrophages and neutrophils	Chemotactically attracts
		Hepatocytes	Induces synthesis of acute-phase proteins
		Hypothalamus	Induces fever
IL-2	Th cells	Antigen-primed Th and CTLs	Induces proliferation, enhances activity
IL-3	Th cells, NK cells	Hematopoietic cells (myeloid)	Supports growth and differentiation
IL-4	Th2 cells	Antigen-primed B cells	Costimulates activation
		Activated B cells	Stimulates proliferation and differentiation, induces class switch to IgE
IL-5	Th2 cells and mast cells	Bone marrow cells	Induces eosinophil differentiation
IL-6	Monocytes, macrophages, Th2 cells, bone marrow stromal cells	Proliferating B cells	Promotes terminal differentiation into plasma cells
		Plasma cells	Stimulates Ab secretion
		Myeloid stem cells	Helps promote differentiation
		Hepatocytes	Induces synthesis of acute-phase proteins
IL-7	Bone marrow, thymic stromal cells	Lymphoid stem cells	Induces differentiation into progenitor B and T cells

(Continued)

Cytokine	Secreted by	Target Cell/Tissue	Activity
IL-8	Macrophages, endothelial cells	Neutrophils	Chemokine, induces adherence to endothelium and extravasation into tissues
IL-10	Th2 cells T_{Reg} cells	Macrophages	Suppresses cytokine production by Th1 cells
IL-11	Bone marrow stroma	Bone marrow	↑ platelet count
IL-12	Macrophages, B cells	Activated CD8+ cells	Acts synergistically with IL-2 to induce differentiation into CTLs
		NK and LAK cells and activated Th1 cells	Stimulates proliferation
IL-13	Th2 cell	B cells	Induces isotype switch to IgE
IL-17	Th17 cells	Fibroblasts, endothelial cells, macrophages	Increases inflammation. Attracts PMNs, induces IL-6, IL-1, TGFβ, TNFα, IL-8
IL-18	Macrophages	IFN-γ synthesis	NK cells, Th cells, IFN-γ synthesis
Interferon-α (type I)	Leukocytes	Uninfected cells	Inhibits viral replication
IL-22	Th17	Endothelium	Stabilizes endothelial barrier, induces secretion of microbials
Interferon-β (type I)	Fibroblasts	Uninfected cells	Inhibits viral replication
Interferon-γ (type II)	Th1, CTLs, NK cells	Macrophages	Enhances activity
		Many cell types	Increases expression of classes I and II MHC
		Proliferating B cells	Induces class switch to IgG2a, blocks IL-4–induced class switch to IgE and IgG1
		Th2 cells	Inhibits proliferation
		Phagocytic cells	Mediates effects important in DTH, treatment for CGD
Transforming growth factor-β	Platelets, macrophages, lymphocytes, mast cells	Proliferating B cells	Induces class switch to IgA
Tumor necrosis factor-α	Macrophages, NK cells	Tumor cells	Has cytotoxic effect
		Inflammatory cells	Induces cytokine secretion, causes cachexia of chronic inflammation

(Continued)

Cytokine	Secreted by	Target Cell/Tissue	Activity
Tumor necrosis factor-β	Th1 and CTL	Tumor cells	Has cytotoxic and other effects, like TNF-α
		Macrophages and neutrophils	Enhances phagocytic activity
Granulocyte colony-stimulating factor (G-CSF)	Macrophages and Th cells	Bone marrow granulocyte precursors	Induce proliferation, used clinically to counteract neutropenia following ablative chemotherapy
Granulocyte– macrophage colony-stimulating factor (GM-CSF)	Macrophages and Th cells	Bone marrow granulocyte and macrophage precursors	Induces proliferation; used clinically to counteract neutropenia following ablative chemotherapy

CYTOKINES AVAILABLE IN RECOMBINANT FORM

Cytokine	Clinical Uses
Aldesleukin (IL-2)	↑ lymphocyte differentiation and ↑ NKs—used in renal cell cancer and metastatic melanoma
Interleukin-11	↑ platelet formation—used in thrombocytopenia
Filgrastim (G-CSF)	↑ granulocytes—used for marrow recovery
Sargramostim (GM-CSF)	↑ granulocytes and macrophages—used for marrow recovery
Erythropoietin	Anemias, especially associated with renal failure
Thrombopoietin	Thrombocytopenia
Interferon-α	Hepatitis B and C, leukemias, melanoma
Interferon-β	Multiple sclerosis
Interferon-γ	Chronic granulomatous disease →↑ TNF

Immunology Practice Questions

CELLS OF THE IMMUNE SYSTEM

1. Isotype switching during B-cell ontogeny dedicates mature B cells to production of a single heavy chain isotype, except in the case of IgM and IgD, which can be expressed concomitantly. How is this expression of both isotypes simultaneously possible?

   (A) Allelic exclusion
   (B) Allelic codominance
   (C) Affinity maturation
   (D) Alternative RNA splicing
   (E) Somatic hypermutation

2. A 4-year-old Caucasian boy is brought to his pediatrician with complaints of abnormal bruising and repeated bacterial infections. A blood workup reveals thrombocytopenia and neutropenia and the presence of numerous small, dense lymphoblasts with scant cytoplasm. Immunophenotyping of the abnormal cells determines them to be extremely primitive B cells, which are CD19+, HLA-DR+, and Tdt+. Which of the following best describes the status of immunoglobulin chain synthesis most likely in these cells?

   (A) IgM monomers inserted in the membrane
   (B) IgM monomers present in the cytoplasm
   (C) Mu (μ) chains inserted in the membrane
   (D) Mu (μ) chains present in the cytoplasm
   (E) No immunoglobulin chain synthesis present

3. A young woman with acute myeloblastic leukemia is treated with intensive chemotherapy and achieves remission of her symptoms. Because the prognosis for relapse is relatively high, a bone marrow transplant is undertaken in her first remission. Which of the following cytokines administered with the bone marrow cells would have the beneficial result of stimulating lymphoid-cell development from the grafted stem cells?

   (A) Interleukin (IL)-1
   (B) IL-2
   (C) IL-3
   (D) IL-6
   (E) IL-7

4. A 2-year-old boy is evaluated for a severe combined immunodeficiency disease. His bone marrow has normal cellularity. Radioactive tracer studies demonstrate a normal number of T-cell precursors entering the thymus, but no mature T lymphocytes are found in the blood or peripheral organs. Cells populating the thymus are found to lack CD3. Which of the following capabilities would his cells lack?

   (A) Ability to bind cell-bound peptides
   (B) Ability to express CD4/CD8 coreceptors
   (C) Ability to produce terminal deoxyribonucleotidyl transferase
   (D) Ability to proliferate in response to specific antigen
   (E) Ability to rearrange T-cell receptor gene segments

5. A patient with advanced metastatic melanoma decides to join an experimental treatment protocol in the hope that it will cause regression of his tumor masses. Malignant cells are aspirated from several of his lesions and transfected in vitro with the gene encoding IL-3 production. The transfected tumor cells are then reinfused into the patient. Mobilization of which of the following cells from the bone marrow would be likely to result from this treatment?

   (A) Antigen-presenting cells
   (B) B lymphocytes
   (C) NK cells
   (D) Plasma cells
   (E) T lymphocytes

THE SELECTION OF LYMPHOCYTES

6. An 8-year-old boy is diagnosed with acute lymphoblastic leukemia. Flow cytometry is used to determine the immunophenotype of the malignant cells. The patient's cells are evaluated with monoclonal antibodies for MHC class II, CD19, and CD34, and are found to have high levels of fluorescence with all of these markers. They also possess cytoplasmic μ heavy chains. What is the developmental stage of these cells?

   (A) Immature B cell
   (B) Lymphoid progenitor cell
   (C) Mature B cell
   (D) Pre-B cell
   (E) Pro-B cell

7. The blood from an 8-year-old boy was analyzed by flow cytometry. The cells were treated with fluorescent-labeled antibodies to various cell surface markers before they were evaluated by flow cytometry. Which of the following markers would identify the B lymphocytes in the sample?

   (A) CD3
   (B) CD4
   (C) CD8
   (D) CD19
   (E) CD56

8. An 18-year-old member of a college soccer team is seen by a physician because of chest tightness and dyspnea on exertion. A 15-cm mediastinal mass is detected radiographically. Eighty percent of the white blood cells in the peripheral blood are small, abnormal lymphocytes with lobulated nuclei and scant cytoplasm. Immunophenotyping of the abnormal cells shows them to be CD4+ and CD8+. Where would such cells normally be found in the body?

   (A) Bone marrow
   (B) Peripheral blood
   (C) Thymic cortex
   (D) Thymic medulla
   (E) Splenic periarteriolar lymphoid sheaths

9. A 12-year-old child is diagnosed with a T-cell lymphoma. The phenotype of the malignant cell matches that of normal progenitor cells that leave the bone marrow to enter the thymus. What cell surface markers would you expect to find on the malignant cells?

   (A) CD4–, CD8–, TCR–
   (B) CD4–, CD8–, TCR+
   (C) CD4–, CD8+, TCR+
   (D) CD4+, CD8–, TCR+
   (E) CD4+, CD8+, TCR+

10. Herpes simplex viruses are extremely successful pathogens because they have a variety of immunologic evasion mechanisms. For example, both HSV 1 and 2 depress the expression of MHC class I molecules on the surface of infected cells. Which coreceptor's binding would be inhibited by this technique?

   (A) CD2
   (B) CD4
   (C) CD8
   (D) CD16
   (E) CD56

11. A patient with a B-cell lymphoma is referred to an oncology clinic for the analysis of his condition. The malignant cells are found to be producing IgM monomers. Which of the following therapeutic regimens is most likely to destroy the malignant cells and no others?

(A) Anti-CD3 antibodies plus complement
(B) Anti-CD19 antibodies plus complement
(C) Anti-CD20 antibodies plus complement
(D) Anti-idiotype antibodies plus complement
(E) Anti-μ chain antibodies plus complement

LYMPHOCYTE RECIRCULATION AND HOMING

12. A lymph node biopsy of a 6-year-old boy shows markedly decreased numbers of lymphocytes in the paracortical areas. Analysis of his peripheral blood leukocytes is likely to show normal to elevated numbers of cells expressing surface

(A) CD2
(B) CD3
(C) CD4
(D) CD8
(E) CD19

13. A 65-year-old woman was involved in an automobile accident that necessitated the removal of her spleen. To which of the following pathogens would she have the most increased susceptibility?

(A) *Babesia microti*
(B) *Bordetella pertussis*
(C) *Corynebacterium diphtheriae*
(D) Enteroaggregative *Escherichia coli*
(E) Human papilloma virus

14. A 6-year-old child is taken to his pediatrician because the parents are alarmed about an indurated fluctuant mass on the posterior aspect of his neck. The mass is nontender and shows no signs of inflammation. The child is examined carefully, and no other masses are found. The pediatrician decides to submit a biopsy of this area to a pathologist. The pathologist reports back that the mass is a lymph node with markedly increased numbers of cells in the cortical area. Fluorescent antisera to which of the cell surface markers is most likely to bind to cells in this area?

(A) CD2
(B) CD3
(C) CD4
(D) CD16
(E) CD19

15. A radioactive tracer dye is injected subcutaneously into the forearm of an experimental subject. What is the first area of the first draining lymph node that would develop significant radioactivity?

(A) Cortex
(B) Medulla
(C) Paracortex
(D) Primary follicle
(E) Subcapsular sinus

THE FIRST RESPONSE TO ANTIGEN

16. A rabbit hunter in Arkansas is diagnosed with ulceroglandular tularemia and treated with streptomycin. Within a week, he returns to the hospital. The tularemic papule, lymphadenopathy, and bacteremia have resolved, but he has now developed a raised, itching skin rash and a fever. The drug was discontinued, and the symptoms subsided. What was the role of streptomycin in this case?

(A) It acted as a B-cell mitogen
(B) It acted as a hapten
(C) It acted as a provider of costimulatory signals
(D) It acted as a superantigen
(E) It acted as an immunogen

17. A 2-year-old child who has suffered recurrent bacterial infections is evaluated for immunologic deficiency. The child has age-normal numbers of CD19+ and CD3+ cells in the peripheral blood and an extreme neutrophilia. The nitroblue tetrazolium dye reduction test is normal. What is the most likely defect in this child?

(A) Absence of CCR4
(B) Absence of CD18
(C) Absence of interleukin-1
(D) Absence of interleukin-4
(E) Absence of tumor necrosis factor-α

18. A 2-year-old boy is admitted to the hospital for workup of a possible immunologic disorder. His history is remarkable for the occurrence of multiple skin infections involving *Staphylococcus, Pseudomonas,* and *Candida*. On examination the child has cervical lymphadenopathy and mild hepatosplenomegaly. Blood tests reveal an elevated erythrocyte sedimentation rate and neutrophilia. The nitroblue tetrazolium dye reduction test and neutrophil oxidative index are negative. What is the most likely defect in this child?

(A) C3 deficiency
(B) Deficiency of CD18
(C) Deficiency of myeloperoxidase
(D) NADPH oxidase deficiency
(E) Phagocyte granule structural defect

19. It has been learned in animal experiments that there are advantages to eliciting nonspecific inflammation at the site of inoculation of antigen toward the ultimate development of a protective immune response to that immunogen. Which of the following substances, if introduced with a vaccine, would serve the purpose of attracting a neutrophilic infiltrate into the area?

(A) Complement component 3b
(B) Immunoglobulin G
(C) Interleukin-8
(D) Myeloperoxidase
(E) Tumor necrosis factor-α

THE PROCESSING AND PRESENTATION OF ANTIGEN

20. Human infections with *Mycobacterium leprae* express a spectrum of clinical presentations depending on the extent and expression of their immune response to the intracellular organism. On one end of the spectrum, patients with tuberculoid leprosy produce an effective cell-mediated immune response, which is successful at killing the intracellular organisms and, unfortunately, produces tissue damage. Patients with tuberculoid leprosy have granulomas that have elevated amounts of IL-2, IFN-γ, and TNF-β. The immune cell responsible for this pattern of cytokine production is the

(A) Cytotoxic T lymphocyte
(B) Epithelioid cell
(C) Macrophage
(D) Th1 cell
(E) Th2 cell

21. There is evidence that the immunologic pathway that distinguishes the selection between the two polar forms of leprosy depends on the initial means of antigen presentation, as well as individual human differences in response. If early events of antigen recognition elicit production of IL-4, IL-5, IL-6, and IL-10, lepromatous leprosy is more likely to result, with the outcome of failure to mount a protective delayed-type hypersensitivity response. What differential characteristic of the lepromatous form is predicted based on the fact of overproduction of IL-4, IL-5, IL-6, IL-10, IL-13 and TGFβ in lepromatous lesions?

(A) Autoimmunity
(B) Granuloma formation
(C) Hypergammaglobulinemia
(D) Immediate hypersensitivity
(E) Inflammation

22. An elderly man with diabetes develops a blister on the heel of his foot, which becomes infected. Although nursing staff in the home where he is a resident clean and treat the wound with topical antibiotic ointment, he develops a fever and hypotension, and a desquamating rash spreads from the site of the original blister. How does the toxin responsible for his symptoms cause these signs?

    (A) It acts as an IL-1 homologue
    (B) It activates B lymphocytes polyclonally
    (C) It activates complement
    (D) It cross-links MHC class II molecules to TCRs polyclonally
    (E) It stimulates neutrophils

23. It has been learned in several experimental systems that proliferation and differentiation of T lymphocytes in response to tumor cells is low because tumor cells lack the necessary costimulatory molecules for lymphocyte activation. If melanoma cells from a patient were induced to express these costimulatory molecules by transfection, production of an effective antitumor response might occur. Which of the following molecules would be the best candidate for transfection of tumor cells to achieve this end?

    (A) B7
    (B) CD2
    (C) CD4
    (D) CD28
    (E) LFA-1

24. A 50-year-old woman with severe rheumatoid arthritis is started on infliximab (anti-tumor necrosis factor-alpha). This therapy has been shown to increase the production of CD25-positive T cells. Which of the following is likely, therefore, to become elevated in this patient?

    (A) Interferon-gamma
    (B) Interleukin-1
    (C) Interleukin-2
    (D) Interleukin-10
    (E) Transforming growth factor-beta

THE GENERATION OF HUMORAL EFFECTOR MECHANISMS

25. An antibody preparation is being used in a laboratory protocol to study B lymphocytes. The preparation does not activate the cells or cause capping. It does not cause precipitation of its purified ligand, and it does not cause agglutination of latex beads covalently coupled to its ligand. Which of the following is the most likely antibody preparation?

    (A) Monoclonal anti-CD19 IgG
    (B) Monoclonal anti-CD56 IgG
    (C) Papain-treated anti-CD19 IgG
    (D) Papain-treated anti-CD56 IgG
    (E) Pepsin-treated anti-CD19 IgG
    (F) Pepsin-treated anti-CD56 IgG

26. IgM isohemagglutinins from an individual of blood group A are treated with pepsin. When the product of this reaction is added to group B erythrocytes, they will be

    (A) agglutinated
    (B) lysed
    (C) phagocytized
    (D) precipitated
    (E) unaffected

27. A 26-year-old obstetric patient becomes ill during the first trimester of pregnancy with fever and lymphadenopathy. She is found to have a rising titer of anti-*Toxoplasma gondii* antibodies. She delivers a full-term baby with no apparent signs of in utero infection. The best test to diagnose acute infection in the neonate would be a parasite-specific ELISA for which isotype of immunoglobulin?

    (A) IgA
    (B) IgD
    (C) IgE
    (D) IgG
    (E) IgM

28. A 4-year-old boy is evaluated for a possible immunologic deficiency. He has suffered repeated infections of mucosal-surface pathogens and has shown delayed development of protective responses to the standard childhood vaccinations. Immunoelectrophoresis of his serum demonstrates absence of a macroglobulin peak, and his sputum is devoid of secretory IgA. Normal numbers of B lymphocytes bearing monomeric IgM are found by flow cytometry, and serum levels of monomeric IgA, IgE, and each of the 4 subisotypes of IgG are normal. Which of the following deficiencies could account for these findings?

(A) Absence of CD40
(B) Absence of J chains
(C) Absence of IL-4
(D) Absence of Tdt
(E) Absence of Th2 cells

29. A 56-year-old homeless, alcoholic, and febrile man is brought to the emergency department after a difficult night during which his coughing kept everyone at the shelter awake. On arrival his pulse is rapid, and his breathing is labored with diffuse rales. Endotracheal aspirates produce a mucopurulent discharge containing numerous gram-positive cocci in chains. His serum contains high titers of IgM antibodies specific for the polysaccharide capsule of *Streptococcus pneumoniae*. The effector mechanism most likely to act in concert with this early IgM production to clear infection is

(A) ADCC
(B) complement-mediated opsonization
(C) cytotoxic T lymphocytes
(D) LAK cells
(E) NK cells

30. A 3-year-old boy has had several bouts with pneumonia. *Streptococcus pneumoniae* was isolated and identified in each of the cases. The child was treated with penicillin each time, and the condition resolved. He is now being evaluated for a potential immunologic deficiency. Serum electrophoresis shows age-normal values for all isotypes of immunoglobulin, but serum levels of some components of complement are depressed. Which of the following deficiencies could explain his problem?

(A) C1
(B) C2
(C) C3
(D) C4
(E) C5

31. Up until the 1970s, tonsillectomies were routinely performed on children with swollen tonsils. This procedure has lost its widespread appeal as we have learned the important role of mucosal-associated lymphoid tissue (MALT) in the protective immune response. What is the major immunoglobulin produced by the MALT?

   (A) A dimeric immunoglobulin with secretory component
   (B) A monomeric immunoglobulin that crosses the placenta
   (C) A monomeric immunoglobulin bound by mast cells
   (D) A monomeric immunoglobulin that opsonizes
   (E) A pentameric immunoglobulin that activates complement

32. A 64-year-old man undergoes surgery to excise 18 inches of bowel with adenocarcinoma. When the tissue and draining mesenteric lymph nodes are sent for pathologist's examination, the Peyer patches are noted to be hyperplastic with IgA-secreting plasma cells, but there is no secretory IgA found in the lumen of the colon. Which of the following changes in the bowel epithelium could explain this finding?

   (A) Failure of isotype switching
   (B) Failure of variable domain gene-segment rearrangement
   (C) Loss of J chain synthesis
   (D) Loss of the polyimmunoglobulin receptor
   (E) Loss of Th2 cells

THE GENERATION OF CELL-MEDIATED EFFECTOR MECHANISMS

33. A 62-year-old accountant develops a solid tumor that is unresponsive to chemotherapy. He elects to participate in an experimental treatment protocol to stimulate his own immune effector cells to recognize and kill the malignant cells. The tumor cells are found to have no expression of MHC class I antigens. Which of the following in vitro treatments of his tumor cells is likely to stimulate the most effective immune response when reinfused into the patient?

   (A) IFN-γ
   (B) IL-2
   (C) IL-8
   (D) IL-10
   (E) TNF-β

34. *Toxoplasma gondii* is an intracellular parasite that lives inside phagocytic and nonphagocytic cells by generating its own intracellular vesicle. This may allow it to avoid recognition and killing by CD8+ lymphocytes, which require the presentation of foreign peptides transported into the endoplasmic reticulum and loaded onto MHC molecules that have

(A) a β_2 domain instead of a β_2 microglobulin
(B) invariant chains
(C) a peptide-binding groove
(D) a single transmembrane domain
(E) two similar chains

35. Before 1960, children with enlarged thymus glands were frequently irradiated to functionally ablate this organ, whose role was not yet known. Over the lifetime of such individuals, which of the following conditions was likely to develop?

(A) Depressed immune surveillance of tumors
(B) Depressed oxygen-dependent killing by neutrophils
(C) Depressed primary response to soluble antigens
(D) Increased cellularity of lymph node paracortical areas
(E) Increased tendency toward atopy

36. A 42-year-old Nigerian man who is in the United States visiting with his brother comes into the hospital clinic. He complains of several months of weight loss, night sweats, mild sputum production, and the spitting up of blood. You run a PPD skin test and the results are positive. What can you conclude from this result?

(A) A cell-mediated immune response has occurred
(B) A humoral immune response has occurred
(C) The B-cell system is functional
(D) The B- and T-cell systems are functional
(E) The neutrophilic phagocyte system is functional

37. A woman with advanced metastatic breast cancer undergoes a radical mastectomy, followed by irradiation and chemotherapy. After a 2-year remission, a metastatic focus appears, and she enrolls in an experimental treatment protocol. In it, a sample of her aspirated bone marrow is treated with GM-CSF, TNF-α, and IL-2, then pulsed with membrane fragments of her tumor cells and reinfused. Which of the following cell subpopulations is the most directly targeted by this treatment?

(A) B lymphocytes
(B) Cytotoxic T lymphocytes
(C) NK cells
(D) Th1 cells
(E) Th2 cells

THE GENERATION OF IMMUNOLOGIC MEMORY

38. In a lifetime, a person may receive a dozen or more tetanus toxoid inoculations. When boosters are administered at 10-year intervals, which of the following would be true of the B lymphocytes that respond?

    (A) Their receptors would have high avidity
    (B) They would be large and highly metabolic
    (C) They would have low levels of adhesion molecules
    (D) They would have surface IgG, IgA, or IgE
    (E) They would have surface IgM

VACCINATION AND IMMUNOTHERAPY

39. A 10-year-old child was bitten by a stray dog. The child is started on a course of anti-rabies post-exposure prophylaxis, beginning with inoculation of pooled human antirabies immunoglobulin. What would repeated inoculation of this antirabies immunoglobulin preparation be likely to induce?

    (A) Anti-allotype antibodies
    (B) Anti-epitope antibodies
    (C) Anti-idiotype antibodies
    (D) Anti-isotype antibodies
    (E) Anti-rabies antibodies

40. All residents of a Chicago nursing home are inoculated intramuscularly with an H3N2 influenza A preparation. The goal of this protocol is to stimulate which of the following types of immunity?

    (A) Adaptive
    (B) Artificial active
    (C) Artificial passive
    (D) Natural active
    (E) Natural passive

41. A city sanitation worker is struck by a car and his leg is crushed against his sanitation truck. The extreme trauma to the leg necessitates amputation above the knee. Although the patient's health records reflect a tetanus booster 6 years ago, the man is revaccinated and human, pooled antitetanus immunoglobulin is injected around the macerated tissue. Administration of immunoglobulin is an example of which of the following forms of immunization?

    (A) Adaptive
    (B) Artificial active
    (C) Artificial passive
    (D) Natural active
    (E) Natural passive

42. A 28-year-old man was brought into court for nonpayment of child support. A 20-year-old woman insists that he is the father of her child. The court suggests before hearing the paternity case that various genetic tests be performed on the man, woman, and child. One of the sets of tests was for genetic immunoglobulin identification. Which immunoglobulin marker would be useful in this case?

(A) Allotype
(B) Idiotype
(C) IgA2
(D) IgM
(E) Isotype

43. In 1988 a new childhood vaccine was developed to protect against epidemic meningitis by mixing *Haemophilus influenzae* type B capsular polysaccharide with whole, killed *Bordetella pertussis* bacteria. The function of the whole, killed bacteria in this vaccine was as a(n)

(A) carrier
(B) hapten
(A) mitogen
(D) adjuvant
(E) immunogen

IMMUNODEFICIENCY DISEASES

44. A newborn is evaluated for immunologic function. He has a distortion of the shape of his mouth, low-set and malformed ears, and widely spaced eyes. Radiographically, there is evidence of cardiac malformation and absence of a thymic shadow. Which of the following parameters would be normal in this child?

(A) Antibody-dependent cell-mediated cytotoxicity of parasite targets
(B) Cellularity of splenic periarteriolar lymphoid sheaths
(C) Cytotoxic killing of virus-infected targets
(D) Generation of oxygen metabolites in phagocytic cells
(E) Proliferative response to concanavalin A

45. A 14-month-old boy is referred to a specialist for diagnosis of a potential immunologic deficiency. For the past 4 months, the child has suffered repeated episodes of bacterial infections and attempts to induce immunity using the pneumococcal vaccine have failed. Studies of peripheral blood indicate an absence of cells responsive to pokeweed mitogen. Bone marrow aspirates are remarkable for hypercellularity of pre-B cells. What is the most likely diagnosis?

    (A) Bruton agammaglobulinemia
    (B) Common variable hypogammaglobulinemia
    (C) DiGeorge syndrome
    (D) Selective immunoglobulin deficiency
    (E) Wiskott-Aldrich syndrome

46. A 31-year-old man is treated for a fourth episode of disseminated *Neisseria gonorrhoeae* infection in the last 5 years. He had no previous history of unusual or recurrent infections. If he has an immunologic defect, which of the following is most likely?

    (A) Common variable immunodeficiency
    (B) C8 deficiency
    (C) DiGeorge syndrome
    (D) Selective IgA deficiency
    (E) Severe combined immunodeficiency

47. A patient has been hospitalized 3 times for painful abdominal edema and is complaining now of swollen lips. What will laboratory findings in this patient most likely include?

    (A) Abnormal superoxide anion production by neutrophils
    (B) Abnormal T-cell function
    (C) Abnormal T-cell numbers
    (D) Defective neutrophil chemotaxis
    (E) Reduced C4 levels

48. A 4-year-old girl presents with a severe *Staphylococcus aureus* abscess. Her history is significant for a previous infection with *Serratia marcescens*. If she has an enzyme deficiency, which of the following is most likely?

    (A) Adenosine deaminase
    (B) C1 inhibitor
    (C) Myeloperoxidase
    (D) NADPH oxidase
    (E) Superoxide dismutase

49. An acutely ill, 2-year-old boy is hospitalized with *Staphylococcus aureus* pneumonia, which is treated appropriately. The patient's history indicates similar bouts of bacterial infections in the past. He had recovered uneventfully from measles 6 months ago. Physical examination discloses scant tonsillar tissue and no palpable lymphadenopathy. Immunoelectrophoresis reveals subnormal levels of gammaglobulins. The nitroblue tetrazolium and chemiluminescence assays indicate normal phagocytic killing. Which of the following disorders is most likely responsible for this child's condition?

(A) Adenosine deaminase deficiency
(B) Defect of the *Btk* gene
(C) Defect of the *SAP* gene
(D) Defect of the *WAS* gene
(E) ICAM-1 deficiency

50. A 2-year-old boy suffering from repeated painful bouts of inflammation of mucosal surfaces, especially affecting the lips, is brought to the pediatrician's office. The mother remembers similar symptoms in previous generations of her family and fears a heritable tendency toward food allergy. What laboratory finding would best support the physician's suspicion?

(A) Depressed C3
(B) Depressed C4
(C) Depressed C5
(D) Elevated C1
(E) Elevated C1, C4, and C2

51. A 10-month-old infant girl is admitted to the hospital with signs of *Pneumocystis jirovecii* pneumonia. Studies of her peripheral blood demonstrate age-normal counts of CD19+ cells, but CD3+ and CD4+ cell numbers are depressed. Immunoelectrophoresis of her serum reveals a moderate hypogammaglobulinemia. Her peripheral blood lymphocytes proliferate normally in response to phytohemagglutinin and MHC class I mismatched allogeneic cells. In a one-way mixed lymphocyte reaction using her cells as the stimulator cells, allogeneic T lymphocytes did not proliferate. Which of the following best describes the molecule most likely lacking from her lymphocytes?

(A) It is designed to bind endogenously produced peptides
(B) It is designed to bind exogenously processed peptides
(C) It possesses β_2 microglobulin
(D) It possesses two chains of unequal length
(E) It should be present on all nucleated cells in the body

DISEASES CAUSED BY IMMUNE RESPONSES: HYPERSENSITIVITY AND AUTOIMMUNITY

52. A 36-year-old farmer has been exposed to poison ivy on several different occasions, and he usually gets very severe skin lesions. A pharmaceutical company is developing cytokines by recombinant DNA technology and formulating them in a fashion that they are readily absorbed through the skin. Which of the following cytokines administered topically could inhibit the severity of this reaction?

    (A) γ-Interferon
    (B) IL-2
    (C) IL-3
    (D) IL-8
    (E) IL-10

53. In the 1960s, it was quickly ascertained that Peace Corps workers sent to schistosome-endemic areas were exposed to massive initial doses of cercariae before any protective immunity existed. In these individuals, IgG antibodies developed in response to the developing worms, and when the adults began their prodigious release of eggs into the circulation, the patients suffered acute and potentially life-threatening symptoms of fever, edema, arthralgia, and rash. Which of the following is another condition that arises by a similar immunologic mechanism?

    (A) Arthus reaction
    (B) Atopic allergy
    (C) Goodpasture syndrome
    (D) Tuberculin reaction
    (E) Transfusion reaction

54. In native Egyptian populations, children are exposed to the cercariae of the fluke *Schistosoma mansoni* in early childhood when they wade in irrigation ditches throughout the Nile Delta. On first exposure, the cercariae penetrate the skin and become schistosomula, which enter the circulation and eventually mature in the mesenteric veins. On subsequent exposures, schistosomula are frequently killed within minutes by an immune response in the skin manifested by intense itching, stinging, and urticaria. What is this protective immune response a manifestation of?

    (A) Arthus reaction
    (B) Contact dermatitis
    (C) Passive cutaneous anaphylaxis
    (D) Serum sickness
    (E) Type I hypersensitivity

TRANSPLANTATION IMMUNOLOGY

55. A 42-year-old auto mechanic has been diagnosed with end-stage renal disease. His twin brother is HLA identical at all MHC loci and volunteers to donate a kidney to his brother. What type of graft transplant terminology is correct in this situation?

    (A) Allograft
    (B) Autograft
    (C) Heterograft
    (D) Isograft
    (E) Xenograft

56. A patient with acute myelogenous leukemia (AML) undergoes irradiation and chemotherapy for his malignancy while awaiting bone marrow transplantation from a closely matched sibling. Six months after the transplant, the immune response appears to be reconstituting itself well—until 9 months postinfusion, when symptoms of generalized rash with desquamation, jaundice, and bloody diarrhea begin to appear. A second, more closely matched bone marrow donor is sought unsuccessfully, and 10 months after the transfer, the patient dies. What is the immunologic effector mechanism most closely associated with this rejection reaction?

    (A) Activated macrophages
    (B) Antibodies and complement
    (C) CD8+ lymphocytes
    (D) LAK cells
    (E) NK cells

57. A child who requires a kidney transplant has been offered a kidney by both parents and 3 siblings. A one-way mixed lymphocyte reaction between prospective donors and recipient is performed, and the stimulation indices are shown. The stimulation index is the ratio of proliferation (measured by [^{3}H]-thymidine incorporation) of the experimental group versus the negative control group. Which of the prospective donors would be the best choice?

	Irradiated Stimulator Cells					
Responder Cells	**Recipient**	**Father**	**Mother**	**Sibling 1**	**Sibling 2**	**Sibling 3**
Recipient	1.0	4.1	2.3	1.1	8.3	8.5
Father	5.3	1.0	12.3	5.6	4.9	5.9
Mother	3.2	12.6	1.0	4.5	3.9	4.8
Sibling 1	1.6	6.5	5.5	1.0	4.4	6.0
Sibling 2	7.6	5.9	4.9	4.4	1.0	7.8
Sibling 3	9.0	5.7	4.4	7.0	8.9	1.0

(A) Father
(B) Mother
(C) Sibling 1
(D) Sibling 2
(E) Sibling 3

58. A 6-year-old child from Zimbabwe is admitted to a U.S. oncology center for treatment of an advanced case of Burkitt lymphoma. Analysis of the malignant cells reveals that they are lacking MHC class I antigens on their surface. Which of the following cytokines produced by recombinant DNA technology could be injected into his solid tumor to increase this tumor cell's susceptibility to CD8+-mediated killing?

(A) IFN-γ
(B) IL-1
(C) IL-2
(D) IL-10
(E) TNF-α

LABORATORY TECHNIQUES IN IMMUNOLOGY

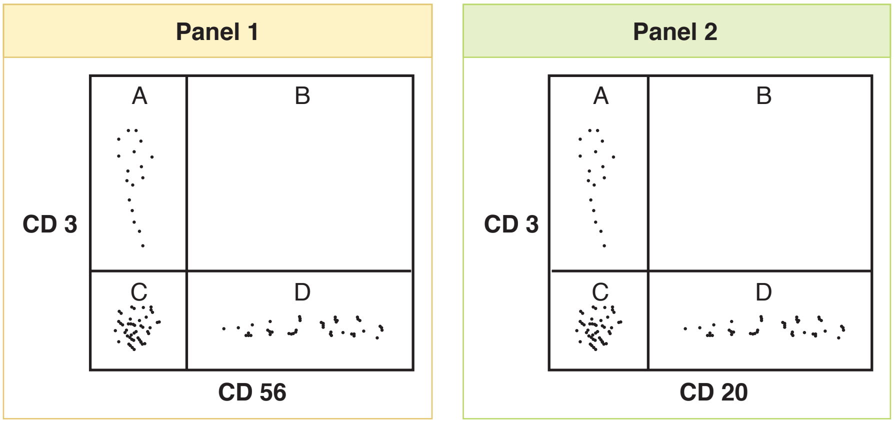
59. A 16-year-old runaway heroin user visits a family planning/STD clinic irregularly to receive birth control pills. In April 2004, the standard HIV screen performed by this clinic reports back that her test was positive. What does the primary test for HIV infection use?

    (A) Electrophoresis of HIV antigens in polyacrylamide gel
    (B) HIV antigen covalently coupled to RBC, patient serum, and anti-immunoglobulin
    (C) HIV antigen covalently coupled to RBC, patient serum, and complement
    (D) HIV antigen, patient serum, anti-immunoglobulin serum, and enzyme-substrate ligand
    (E) HIV antigen, patient serum, anti-immunoglobulin serum, and radioactive ligand

60. A direct Coombs test was performed on a baby in its seventh month of gestation. The mother has had trouble with two earlier pregnancies, and she has never received RhoGAM™. The physician is concerned about the possibility of erythroblastosis fetalis. What ingredients would be necessary to perform this procedure?

    (A) Mother's serum plus RhoGAM plus Coombs reagent
    (B) Mother's serum plus Rh– RBCs plus Coombs reagent
    (C) RhoGAM plus Rh+ RBCs from the baby
    (D) Rh+ RBCs from the baby plus Coombs reagent
    (E) Rh+ RBCs plus mother's serum plus Coombs reagent

61. A patient with Chediak-Higashi syndrome is analyzed for ability to mobilize NK cells into the peripheral blood. His peripheral blood leukocytes are treated with fluorescent-labeled antibodies to CD3, CD56, and CD20 before they are passed through a fluorescence-activated cell sorter. The computer-generated results of this process are shown. In which quadrant of which panel would the natural killer cells be found?



(A) Panel 1, quadrant A
(B) Panel 1, quadrant B
(C) Panel 1, quadrant C
(D) Panel 1, quadrant D
(E) Panel 2, quadrant A
(F) Panel 2, quadrant B
(G) Panel 2, quadrant C
(H) Panel 2, quadrant D

62. In both ABO blood typing and the Coombs test for detection of hemolytic disease of the newborn, agglutination of coated erythrocytes is a positive test result. Why is addition of Coombs reagent not a necessary step in ABO blood typing?

(A) All antibodies made in response to blood glycoproteins are IgG
(B) Complement-mediated lysis is not important in ABO incompatibilities
(C) Coombs serum identifies only anti-Rh antibodies
(D) IgM pentamers are large enough to agglutinate erythrocytes directly
(E) The high titer of natural isohemagglutinins makes Coombs reagent unnecessary

63. A young woman is in the care of an infertility specialist for evaluation of her inability to conceive since her marriage 5 years ago. As a first step in her examination, cervical scrapings are tested for the possibility of undiagnosed infection with *Chlamydia trachomatis*, which could cause fallopian tube scarring. Which of the following diagnostic tests could be used to identify chlamydial antigens in this specimen?

(A) Direct fluorescent antibody test

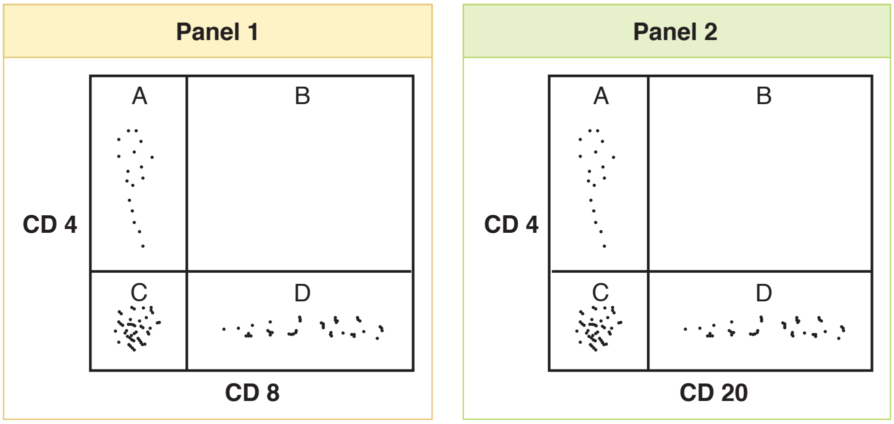
(B) Enzyme-linked immunosorbent assay (ELISA)

(C) Indirect fluorescent antibody test

(D) Radioimmunoassay

(E) Western blot

64. An experimental treatment for melanoma involves in vitro stimulation of tumor-specific killer cells with tumor cells transfected with a gene for production of altered-self MHC class I molecules. As a first step, peripheral blood leukocytes from the patient are incubated with fluorescent-labeled antibodies against CD4, CD8, and CD20. The cells are then subjected to flow cytometry and separated into different populations based on their expression of these cell surface markers. In which quadrant would you find the cell subpopulation most likely to produce a beneficial anti-tumor response in this protocol?



(A) Panel 1, quadrant A

(B) Panel 1, quadrant B

(C) Panel 1, quadrant C

(D) Panel 1, quadrant D

(E) Panel 2, quadrant A

(F) Panel 2, quadrant B

(G) Panel 2, quadrant C

(H) Panel 2, quadrant D

ANSWERS AND EXPLANATIONS

1. **The correct answer is D.** Alternative RNA splicing allows a mature B cell to attach either δ or μ constant domains on a single idiotype that has been generated by germ-line DNA rearrangements.

   Allelic exclusion (**choice A**) refers to the expression of products of either parental chromosome type, but not both. This allows lymphoid cells to express only one type of antigen receptor (one idiotype) per cell and is essential to cellular specificity of action.

   Allelic codominance (**choice B**) refers to the expression of products of both parental chromosomes simultaneously. It is found in the expression of MHC class I and II products, but not in the expression of antigen receptors.

   Affinity maturation (**choice C**) refers to the increase of affinity (binding strength) of a population of antibodies over time during the development of an immune response. Because the affinity of an antibody is dependent on the goodness-of-fit of its idiotype for its antigen, isotype switching does not affect the shape of the idiotype and does not change the affinity of the molecule.

   Somatic hypermutation (**choice E**) is the phenomenon that allows affinity maturation to occur. It is the accelerated mutation of DNA coding within the hypervariable region that occurs during B-cell proliferation in response to antigenic stimulation. Again, the isotype of the antibody does not affect the shape of the idiotype, and this term refers to a process that changes the shape of the idiotype.

2. **The correct answer is E.** This child has acute lymphoblastic leukemia (ALL), and the malignant cells have the characteristics of early B-cell precursors. This leukemia has peak incidence at approximately 4 years of age, is twice as common in whites than in non-whites, and is slightly more frequent in boys than in girls. A leukemic cell that is positive for terminal deoxyribonucleotidyl transferase (Tdt) is in the process of rearranging the gene segments for synthesis of the heavy chain of immunoglobulin but will not yet have completed a functional product. Tdt is active for all heavy-domain gene segment rearrangements but is not used during light-chain gene segment rearrangements.

   IgM monomers inserted in the membrane (**choice A**) would be found in leukemic cells that are at the mature B-cell stage. Such cells would have completed the rearrangements for both heavy and light chains and would lack Tdt as a marker. They would express surface MHC class II, CD19, and CD20 in addition to surface immunoglobulin.

   IgM monomers present in the cytoplasm (**choice B**) would be found in cells that have completed the rearrangement of their variable domain gene segments. They would no longer express Tdt.

   Mu (μ) chains inserted in the membrane (**choice C**) would be found in cells that have completed the rearrangement of their heavy chain variable domain gene segments, and these may transiently be expressed on the surface of a cell in association with a surrogate light chain before light chain rearrangement is complete. These cells would not be using their Tdt anymore.

   Mu (μ) chains in the cytoplasm (**choice D**) would be found in leukemic cells that are more highly differentiated than those described. Once the variable domain gene segments for the heavy chain have been successfully rearranged

in a cell, μ chains can be found in the cytoplasm. In ALL, this is usually associated with a decreased expression of Tdt and appearance of CD10 (the common acute lymphoblastic leukemia antigen; CALLA) and CD20.

3. **The correct answer is E.** The cytokine most strongly associated with stimulation of production of lymphoid cells from the bone marrow is interleukin (IL)-7.

   IL-1 (**choice A**) is the endogenous pyrogen. It is produced by macrophages and acts on the hypothalamus to raise the temperature set point. It is associated with systemic inflammatory processes, but is not known to have an effect on lymphopoiesis.

   IL-2 (**choice B**) is a product of T cells that stimulates proliferation of T cells in the periphery. It is not known to have an effect on lymphopoiesis.

   IL-3 (**choice C**) is the cytokine that is most strongly associated with stimulation of myeloid cell precursors in the bone marrow.

   IL-6 (**choice D**) is a second endogenous pyrogen. It causes production of acute-phase proteins from hepatocytes and acts on myeloid stem cells in the bone marrow to induce differentiation.

4. **The correct answer is D.** CD3 is the signal transduction complex in T lymphocytes. When specific antigen binding has occurred on the surface of the cell, this complex is responsible for transferring the message to the cytoplasm of the cell. This culminates in intracytoplasmic phosphorylation events, which activate the cell and induce its proliferation (cloning). A cell lacking CD3 would be capable of binding specific antigen, but incapable of activation and proliferation in response to that first signal.

   Ability to bind cell-bound peptides (**choice A**) would not be affected by the absence of CD3. Binding to peptides presented by antigen-presenting cells is through interaction of the T-cell receptor with major histocompatibility antigens on the surface of other cells.

   Ability to express coreceptors (**choice B**) would not be affected by the absence of CD3, although cells would not be able to complete their differentiation in the thymus and become fully committed T cells.

   Ability to produce terminal deoxyribonucleotidyl transferase (**choice C**) would not be affected by the absence of the T-cell signal transduction complex. T-cell precursors rearrange their receptor gene segments (and use terminal deoxyribonucleotidyl transferase) in the absence of antigenic stimulation and before signal transduction through CD3 becomes critical.

   Ability to rearrange T-cell receptor gene segments (**choice E**) would not be affected by the absence of the T-cell signal transduction complex. T-cell precursors rearrange their receptor gene segments in the absence of antigenic stimulation and before signal transduction through CD3 becomes critical.

5. **The correct answer is A**. Tumor cells transfected with the gene encoding IL-3 would produce IL-3. This is a cytokine that acts on the bone marrow to cause production and mobilization of myeloid cells. The goal of such therapy would be to induce the production of antigen-presenting cells, which might increase the presentation of tumor-cell antigens to cells important in cell-mediated cytotoxicity.

B lymphocytes (**choice B**) would not be mobilized by such a treatment. The cytokine that favors development of lymphoid precursors in the bone marrow is IL-7.

NK cells (**choice C**) would not be mobilized by such a treatment. Although NK cells are granular, they are derived from lymphoid, not granulocyte/monocyte, precursors. The cytokine that favors development of lymphoid precursors in the bone marrow is IL-7.

Plasma cells (**choice D**) are produced in the secondary lymphoid organs and submucosa. IL-7, which stimulates lymphoid precursors in the bone marrow, would have an indirect effect on plasma cell production, but they are not mobilized from the bone marrow.

T lymphocytes (**choice E**) would not be mobilized by such a treatment. The cytokine that favors development of lymphoid precursors in the bone marrow is IL-7.

6. **The correct answer is D.** The leukemic cells are pre-B cells. They have rearranged their immunoglobulin genes to encode a μ heavy chain. MHC class II antigens are expressed beginning at the pro-B cell stage, as are CD19 and CD20. CD34 is a marker for early lymphohematopoietic stem and progenitor cells, and it functions as a cell–cell adhesion molecule. These cells would also have expressed CD10, the common acute lymphoblastic leukemia antigen (CALLA), which functions as a metalloendopeptidase.

   Immature B cells (**choice A**) have accomplished both and heavy and light immunoglobulin chain rearrangements and therefore express IgM molecules on their cell surface. They would be Tdt-negative, CD19- and CD20-positive, MHC class II-positive, and CD34-negative.

   Lymphoid progenitor cells (**choice B**) would not have completed any of the gene rearrangements necessary to create an immunoglobulin molecule. They would be Tdt-negative, MHC class II–negative, CD19- and CD20-negative, and CD34-positive.

   Mature B cells (**choice C**) possess surface IgM and IgD molecules and are capable of responding to foreign antigen. They are Tdt-negative, MHC class II-positive, CD19- and CD20-positive, CD34-negative, and may express CD40.

   Pro-B cells (**choice E**) are rearranging their immunoglobulin heavy chain gene segments but have not yet completed the process. Therefore, they have no completed chains either cytoplasmically or on their cell surfaces. They would be positive for Tdt, MHC class II, CD19, and CD20.

7. **The correct answer is D.** The best markers for identification of B lymphocytes are CD19, CD20, and CD21. CD19 and CD21 form a coreceptor complex during B-cell activation. The role of CD20 in B-cell activation is unclear, although it forms a calcium-ion channel. CD21 is also a receptor for the C3d component of complement and the Epstein-Barr virus.

   CD3 (**choice A**) is the signal transduction complex of T cells. It is found on all T cells in association with the T-cell antigen receptor.

   CD4 (**choice B**) is found on all helper T lymphocytes.

   CD8 (**choice C**) is found on all cytotoxic T lymphocytes.

   CD56 (**choice E**) is a marker for human natural killer cells.

8. **The correct answer is C**. This patient has a T-cell lymphoblastic lymphoma. In his case, the malignant cell is "double-positive": it possesses both CD4 and CD8. In a normal individual, these would only be found as an early developmental stage in the cortex of the thymus. Once cells have rearranged their receptor genes and been subjected to positive and negative selection, the cells leaving the thymus will express one coreceptor or the other but never both.

   Bone marrow (**choice A**) would contain T lymphocyte precursors that are double negative: They will lack both CD4 and CD8.

   Peripheral blood (**choice B**) would have mature T cells that have differentiated into either helper (CD4+) or cytotoxic (CD8+) cells. There should be no double-positive T cells in the peripheral blood.

   Thymic medulla (**choice D**) is the location of maturing T cells ready to circulate into the bloodstream and peripheral lymphoid organs. It would have only single-positive cells.

   Splenic periarteriolar lymphoid sheaths (**choice E**) are the T-cell–dependent areas of the spleen. They would have fully committed helper (CD4+) or cytotoxic (CD8+) cells.

9. **The correct answer is A.** T-lymphocyte precursors that leave the bone marrow and move to the thymus have neither CD4 nor CD8 coreceptors, and they have not rearranged the DNA of the variable domains of their antigen receptor, the TCR.

   CD4–, CD8–, and TCR+ (**choice B**) is not a possible T-cell phenotype. Once the TCR gene segments are rearranged and the TCR is expressed, the cells will bear both CD4 and CD8 coreceptors.

   CD4–, CD8+, and TCR+ (**choice C**) is the phenotype of cytotoxic T cells that would be in the circulation, not in the thymus, unless it were immediately prior to their release into the circulation following the thymic selection process.

   CD4+, CD8–, and TCR+ (**choice D**) is the phenotype of helper T cells that would be in the circulation, not in the thymus, unless it were immediately prior to their release into the circulation following thymic selection processes.

   CD4+, CD8+, and TCR+ (**choice E**) is the phenotype of cells in the thymic cortex. These are the cells that have rearranged their receptor genes and bear both CD4 and CD8 coreceptors. As the specificity of their TCR is tested, they will be directed to express either CD4 (and become a helper T cell) or CD8 (and become a cytotoxic T cell).

10. **The correct answer is C.** The interaction between the TCR and MHC class I/peptide conjugates is stabilized by the CD8 coreceptor. By downregulating the expression of MHC class I antigens on the surface of infected cells, the virus protects the infected host cell from killing by cytotoxic T lymphocytes.

   CD2 (**choice A**), also known as LFA-2, is an adhesion molecule within the immunoglobulin superfamily of genes. Its ligand is the integrin LFA-3. It is found on T cells and mediates attachment to other lymphocytes and antigen-presenting cells. It does not have a coreceptor role that would impact MHC class I–restricted killing.

CD4 (**choice B**) is the coreceptor that stabilizes the interaction between MHC class II antigens and the TCR. It is thus important for helper T cells, not cytotoxic T cells.

CD16 (**choice D**) is the Fc receptor involved in binding to immune complexes and promoting antibody-dependent cell-mediated cytotoxicity. It is not involved in the MHC class I–restricted killing by cytotoxic T cells.

CD56 (**choice E**) is a cell surface marker found on NK cells. Its function is unknown. However, since NK activity is enhanced in the absence of MHC class I antigen expression, the downregulation of these molecules by herpes simplex 1 and 2 actually makes infected cells more susceptible to the NK cell form of lysis.

11. **The correct answer is D**. Because malignant cells are clonal in origin, all the cells in this patient's lymphoma should be producing IgM monomers of a single idiotype. Treatment with anti-idiotype antibodies plus complement, therefore, would specifically kill only malignant cells, and leave all other B lymphocytes unharmed.

    Anti-CD3 antibodies plus complement (**choice A**) would kill all T lymphocytes in the body. This lymphoma is clearly of B-cell origin because it is bearing IgM monomers.

    Anti-CD19 antibodies plus complement (**choice B**) would kill all B lymphocytes in the body. It would not specifically target malignant cells.

    Anti-CD20 antibodies plus complement (**choice C**) would kill all B lymphocytes in the body. It would not specifically target malignant cells.

    Anti-μ chain antibodies plus complement (**choice E**) would kill all mature and naive B cells and immature B cells that had completed VDJ rearrangement of their heavy chain genes. It would not be specific for malignant cells.

12. **The correct answer is E.** The paracortex of a lymph node is a T-cell–dependent area. If this area is lacking cellularity, then the patient has a deficiency of T lymphocytes. B-lymphocyte numbers could be normal or even elevated. The only B-cell marker on this list is CD19, the marker which is used clinically to enumerate B cells in the body.

    CD2 (**choice A**), also known as LFA-2, is an adhesion molecule found on T cells, thymocytes, and NK cells. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

    CD3 (**choice B**) is found on all T cells. It is also called the "pan-T" cell marker. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

    CD4 (**choice C**) is found on all helper T lymphocytes. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

    CD8 (**choice D**) is found on all cytotoxic T lymphocytes. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

13. **The correct answer is A.** The spleen is the secondary lymphoid organ that is responsible for primary surveillance against blood-borne antigens. *Babesia microti* is an intraerythrocytic parasite of humans, transmitted by

the same vector tick as Lyme disease. Red blood cells (and their parasites) are filtered by the spleen, so splenectomy is a predisposing factor in development of serious disease with this parasite.

Bordetella pertussis (**choice B**) is a mucosal surface pathogen that attaches to the upper airways. Although its toxin becomes blood-borne, the organism itself is confined to the respiratory tree.

Corynebacterium diphtheriae (**choice C**) is a mucosal surface pathogen that attaches to the upper airways. Although its toxin becomes blood-borne, the organism itself is confined to the respiratory tree.

Enteroaggregative *Escherichia coli* (**choice D**) is an organism that causes diarrhea by producing a biofilm-like aggregation of organisms on the surface of the colonic mucosa, which impedes absorption. It is not likely to be a blood-borne pathogen.

Human papilloma virus (**choice E**) produces localized infections in epithelial cells where it is transferred by human-to-human or human-to-fomite contact. It is not likely to be a blood-borne pathogen.

14. **The correct answer is E.** The cortex of lymph nodes is a B-lymphocyte area. Thus, cells in this area would stain with fluorescent antibodies against CD19, the molecule that serves as a portion of the B-cell signal transduction complex. This molecule would be found on all B cells, but would be absent from T cells, macrophages, and NK cells.

    CD2 (**choice A**) is a T-cell marker. T cells will be found in the paracortical areas of lymph nodes.

    CD3 (**choice B**) is a T-cell marker. It is the signal transduction complex of the T cell and will be found on all T cells. T cells will be found in the paracortical areas of lymph nodes.

    CD4 (**choice C**) is a marker for helper T cells. These cells would be found in the paracortical areas of lymph nodes.

    CD16 (**choice D**) is the Fc receptor for IgG antibodies. It would be found on natural killer and phagocytic cells, which would not be numerous in the cortex of the lymph nodes. Phagocytic cells typically are found in the medullary cords.

15. **The correct answer is E.** Lymph nodes are designed to filter tissue fluids. Fluids entering the lymph nodes do so through the afferent lymphatics and are released into the subcapsular sinus. From there, fluids percolate through the cortex, into the medulla, through the medullary cords, and finally exit through the efferent lymphatics in the hilum.

    The cortex (**choice A**) of the lymph node is directly beneath the subcapsular sinus. It would be the second region of the lymph node to be exposed to the radioactive tracer. The cortex is a B-lymphocyte–rich area.

    The medulla (**choice B**) of the lymph node is rich in macrophages. It would not receive the radioactive fluid until it had passed through the cortex and paracortex.

    The paracortex (**choice C**) of the lymph node is a T-cell area. It lies between the cortex and the medulla and thus would receive the radioactive fluid after the cortical areas.

Primary follicles (**choice D**) are found in the cortex of the lymph node. These are areas of active B-lymphocyte proliferation and cloning. They would receive the radioactivity after it left the subcapsular sinus.

16. **The correct answer is B.** Many drug allergies, such as the one described here, are hapten-carrier immune responses. The drug is not large enough by itself to elicit an immune response (it is a hapten), but when it becomes covalently coupled to the body's own proteins (which act as carriers), the combined molecule becomes immunogenic, and a response against one's own tissues is elicited.

    Acting as a B-cell mitogen (**choice A**) is not correct. B-cell mitogens, such as pokeweed mitogen and lipopolysaccharide, cause polyclonal proliferation of B cells and elaboration of IgM antibodies. The drug allergy described here is not a polyclonal response, but a specific anti–altered-self response generated by T and B lymphocytes and production of antibodies.

    Acting as a provider of costimulatory signals (**choice C**) is not correct. The costimulatory signals required to activate B and T lymphocytes include CD28/B7 and CD40/CD40L interactions. These are additional interactions (beyond the specific recognition of antigen) required for the activation of B and T lymphocytes. Although these costimulatory signals would be involved in the evolution of this allergic response, the streptomycin does not serve as a costimulatory signal.

    Acting as a superantigen (**choice D**) is not correct. Superantigens are materials that crosslink the variable β domain of the T-cell receptor and the α-chain of class II MHC molecules. They induce activation of all T cells that express receptors with a particular Vβ domain. The resulting T-cell mitogenesis causes overproduction of T-cell and macrophage cytokines and system-wide pathology. Toxic shock syndrome toxin-1 and *Streptococcus pyogenes* erythrogenic exotoxins act as superantigens.

    Acting as an immunogen (**choice E**) is not correct because streptomycin is not large enough to be immunogenic. Immunogens must be large enough to have at least two epitopes. It is only through binding to a larger carrier protein (the patient's own tissue proteins) that a hapten such as a drug can become immunogenic.

17. **The correct answer is B.** This child has leukocyte adhesion deficiency (LAD), which is a genetic deficiency of CD18. CD18 is an essential component of a number of integrins, and absence of these molecules causes the inability of WBCs to migrate into sites of inflammation. Thus in this patient, the blood contained abnormally high numbers of neutrophils, but they were unable to extravasate. CD18 is a component of LFA-1, CR3, and CR4.

    Absence of CCR4 (**choice A**) would cause difficulties in extravasation and migration of activated T cells and monocytes. This chemokine receptor is not found on neutrophils and therefore would have no effect on neutrophil migration.

    Absence of interleukin-1 (**choice C**) might cause difficulties in producing the acute and chronic inflammatory responses. This cytokine, frequently referred to as the endogenous pyrogen, produces fever, acute phase protein production, and many other results critical to inflammation. However, the actions of IL-1 are extremely redundant with those of IL-6 and tumor necrosis factor-α, so such a condition might have no clinically observable effects.

Absence of interleukin-4 (**choice D**) would result in defects in the ability to mount a normal IgE antibody response. This cytokine also serves as the major stimulus for the development of Th2 cells from naive helper T cells, so its absence would be likely to have profound effects on all aspects of the secondary antibody response.

Absence of tumor necrosis factor-α (**choice E**) might cause difficulties in producing the acute and chronic inflammatory responses. This cytokine has many functions that are redundant with those of IL-1 and IL-6, so such a condition might have no clinically observable effects.

18. **The correct answer is D.** This child has chronic granulomatous disease (CGD). The history indicates he has had recurrent infections with catalase-positive organisms and has a defect in generating oxygen radicals intracellularly in his phagocytic cells (the negative nitroblue tetrazolium test and neutrophil oxidative index). This genetic defect arises from a failure to produce one of the subunits of NADPH oxidase, which makes the individual incapable of producing intracellular oxygen radicals. Redundant intracellular killing mechanisms (myeloperoxidase and lysosomal contents) are still functional in these patients, but when they are infected with catalase-positive organisms, the substrate for myeloperoxidase (hydrogen peroxide) is destroyed, and the only remaining intracellular killing mechanism (lysosomes) is insufficient to protect from infection.

    C3 deficiency (**choice A**) would cause increased susceptibility to pyogenic infections because C3b is an important opsonin that enhances phagocytosis of extracellular organisms. All extracellular bacteria would be included in this list, not simply catalase-positive ones, as mentioned here. The NBT and NOI would not be negative in this case.

    Deficiency of CD18 (**choice B**) is the cause of leukocyte adhesion deficiency (LAD). Because CD18 is the common β chain of the β_2 integrins, its absence compromises leukocyte function antigen (LFA)-1, as well as complement receptors 3 and 4. Patients with LAD suffer recurrent infections with extracellular pathogens (not just catalase-positive ones) because of defective opsonization, mobilization, adhesion, and chemotaxis. The NBT and NOI would be positive.

    Deficiency of myeloperoxidase (**choice C**) results from a deficiency of an important granule enzyme in phagocytic cells. However, because there are so many redundant mechanisms of intracellular killing, these patients generally have mild symptoms or none at all.

    A phagocyte granule structural defect (**choice E**) is responsible for the Chediak-Higashi syndrome. These patients have chemotactic and degranulation defects, lack NK activity, and have partial albinism.

19. **The correct answer is C.** The only substance on the list that is chemotactic for neutrophils is IL-8. Other neutrophil chemotactic factors that might have been mentioned would include C5a, leukotriene B4, and formyl methionyl peptides.

    Complement component 3b (**choice A**) is not chemotactic for neutrophils. It acts as an opsonin, enhancing the phagocytosis of coated particles.

    Immunoglobulin G (**choice B**) is not chemotactic for neutrophils. It acts as an opsonin, enhancing the phagocytosis of coated particles.

Myeloperoxidase (**choice D**) is not chemotactic for neutrophils. It is an enzyme in the lysosomes of phagocytic cells that generates toxic halide radicals intracellularly when exposed to its substrate, hydrogen peroxide.

Tumor necrosis factor-α (**choice E**) is not chemotactic for neutrophils. It is produced by macrophages and is involved with the production of chronic inflammation and cytotoxicity.

20. **The correct answer is D**. IL-2, IFN-γ, and TNF-β are all elaborated by the Th1 cell. TNF-β can also be made by NK cells. In tuberculoid leprosy, the Th1 arm of the immune response is most active, resulting in a protective (but also damaging) cell-mediated response and a dampening of the antibody response. In lepromatous leprosy, the patient has an overabundance of Th2 responses, causing the production of a nonprotective antibody response.

    Cytotoxic T lymphocytes (**choice A**) are an effector cell in the cell-mediated immune response. They do not elaborate many cytokines but produce cytotoxic molecules, which cause the destruction of specific target cells.

    Epithelioid cells (**choice B**) are modified macrophages. They are extremely secretory and may produce IL-1, IL-6, TNF-α, IFN-γ, and GM-CSF. They are prominent in granulomas, and their cytokines would be elevated in a patient with tuberculoid leprosy, but that was not the question.

    Macrophages (**choice C**), once activated, may produce IL-1, IL-6, TNF-α, IFN-γ, and GM-CSF. They are prominent in granulomas, and their cytokines would be elevated in a patient with tuberculoid leprosy, but again, that was not the question.

    Th2 cells (**choice E**) would be elevated during lepromatous leprosy. The cytokines they secrete include IL-4, IL-5, IL-6, IL-10, IL-13 and TGFβ. These cells are stimulators of the humoral immune response.

21. **The correct answer is C.** In lepromatous leprosy, the activation of the Th2 arm of the immune response results in elicitation of those cytokines that stimulate production of antibody (IL-4, IL-5, IL-6, IL-10, IL-13 and TGFβ) and those that inhibit the development of the protective cell-mediated immune response (IL-4 and IL-10). Therefore, hypergammaglobulinemia is a frequent finding in lepromatous leprosy.

    Autoimmunity (**choice A**) may develop after infectious processes, but there is no evidence that stimulation of Th2 cells, by itself, causes autoimmune disease.

    Granuloma formation (**choice B**) would be decreased after exposure to these cytokines. Granulomas are an expression of the delayed-type hypersensitivity response, which is a function of Th1 cells. IL-10 and IL-4 would depress the Th1 response.

    Immediate hypersensitivity (**choice D**) requires sensitized mast cells and IgE antibodies. Although this result could occur in persons predisposed to atopic allergy, it is not the most likely result of stimulation with Th2 cytokines.

    Inflammation (**choice E**) is primarily mediated by substances released during tissue injury (leukotrienes, histamine, etc.) and the cytokines of activated macrophages (IL-1, IL-6, and TNF-α). It is not enhanced by Th2 cytokines.

22. **The correct answer is D**. This patient is showing signs of toxic shock syndrome, caused by infection of the blister with *Staphylococcus aureus* and the resultant elaboration of the exotoxin TSST-1. This toxin acts as a superantigen, cross-linking the variable β region of the TCR to the α chain of the class II MHC molecule. This binds Th cells and APC together without the specificity of antigen recognition, and so clonal proliferation of T cells and production of IFN-γ leads to activation of macrophages. As a result, the macrophages overproduce the cytokines IL-1, IL-6, and TNF-α, which are toxic at high levels.

    It acts as an IL-1 homologue (**choice A**) is not true. IL-1 is produced by macrophages as a result of T-cell activation, but TSST-1 does not itself act as an IL-1 homologue.

    It activates B lymphocytes polyclonally (**choice B**) is not true. TSST-1 acts on Th cells to stimulate macrophage cytokines. It does not have a direct effect on B-cell proliferation.

    It activates complement (**choice C**) is not correct. TSST-1 does not have an effect on complement.

    It stimulates neutrophils (**choice E**) is not correct. Although neutrophils are stimulated during *Staphylococcus aureus* infection and produce IL-1, which causes fever, the mechanism of action of TSST-1 and other superantigens is not through neutrophil activation.

23. **The correct answer is A.** The B7 molecule on antigen-presenting cells binds to the CD28 molecule on T lymphocytes and serves as a costimulatory signal for their activation. If the tumor cells could be induced to express this costimulatory molecule, they would provide the important activating signal to the T cells.

    CD2 (**choice B**) is the molecule on T lymphocytes that binds to LFA-3 on antigen-presenting cells. If the tumor cell were induced to express CD2, it would bind to the complementary structure on macrophages and not activate the T cells.

    CD4 (**choice C**) is the molecule on T lymphocytes that stabilizes the interaction of MHC class II and the TCR. If the tumor cell were induced to express CD4, it would not increase the tumor-specific response.

    CD28 (**choice D**) is the molecule on T cells that binds to B7. If the tumor cell were induced to express CD28, it would bind to the complementary structure on macrophages and not activate the T cells.

    LFA-1 (**choice E**) is the molecule on T cells that binds ICAM-1 on the antigen-presenting cells. If the tumor cells were induced to express LFA-1, it would bind to the complementary structure on macrophages and not activate the T cells.

24. **The correct answer is D.** CD25-positive T_{Reg} cells have been shown to have a role in maintenance of self-tolerance, and therefore, defects in these cells are being blamed in many cases of autoimmune disease. T_{Reg} cells secrete interleukin-10 which is an anti-inflammatory cytokine.

    Interferon-gamma (**choice A**) is a product of Th1 which activates macrophages and amplifies pro-inflammatory pathways in the body. It is not a product of T_{Reg} cells and would cause additional damage in a case of rheumatoid arthritis, so it would not be a logical goal of therapy.

Interleukin-1 (**choice B**) is endogenous pyrogen which is responsible for the setting of the hypothalamic temperature point. It is a product of macrophages which activates Th1 cells, and therefore would be considered a pro-inflammatory cytokine rather than an anti-inflammatory one.

Interleukin-2 (**choice C**) is a product of Th0 and Th1 cells which causes the proliferation of T cells and the effector cells of cell-mediated immunity. Although IL-2 is required for natural T_{Reg} development, it would not be expected to be increased with the therapy mentioned here.

Transforming growth factor-beta (**choice E**) is a product of T cells and macrophages which is required for natural T_{Reg} development, but with this artificial therapy to increase T_{Reg} numbers, it would not be expected to be elevated.

25. **The correct answer is C.** The cell surface marker which is typically used to identify B lymphocytes is CD19. This is a component of the B-cell/signal transduction complex and thus will be found on all B cells. Treatment of IgG with papain yields two monovalent antigen binding (Fab) fragments and destroys the function of the Fc portion of the molecule. Immunoglobulin molecules that are disrupted this way lose their ability to cross-link the receptors on cells, to promote precipitation or agglutination, and to activate cells by providing a first stimulatory signal.

    Monoclonal anti-CD19 IgG **(choice A)** is a divalent antibody molecule that recognizes the signal transduction complex on B cells. Monoclonal antibodies can cross-link cell-surface receptors and cause capping, cell activation, and precipitation. Agglutination is usually accomplished using IgM because a very large molecule is needed to overcome the zeta potential (repulsive charge) of erythrocytes. If IgG is used, a second developing antibody must be added.

    Monoclonal anti-CD56 IgG **(choice B)** is a divalent antibody molecule that recognizes a molecule found on NK cells. Because both arms of the molecule are intact, it is capable of causing capping, cell activation, precipitation, and agglutination if a developing antiserum is added.

    Papain-treated anti-CD56 IgG **(choice D)** would not be used for the study of B lymphocytes because CD56 is a marker for NK cells.

    Pepsin-treated anti-CD19 IgG **(choice E)** is a divalent molecule possessing two Fab fragments joined together ($F[ab']_2$), and a fragmented Fc region. The $F(ab')_2$ portion of the antibody is capable of causing capping, cell activation, precipitation, and, with a developing antiserum, agglutination.

    Pepsin-treated anti-CD56 IgG **(choice F)** is a divalent molecule possessing two Fab fragments joined together ($F[ab']_2$) and a fragmented Fc region. Its specificity is for NK cells. Additionally, the $F(ab')_2$ portion of the antibody is capable of causing capping, cell activation, precipitation, and, with a developing antiserum, agglutination.

26. **The correct answer is A**. Isohemagglutinins are IgM antibodies that will agglutinate the RBCs of individuals with another blood type. They are believed to be made due to exposure to cross-reactive antigens found on the surface of normal gut flora organisms. Thus, a person of blood group A will produce isohemagglutinins that will agglutinate type B cells. If these antibodies are pretreated with pepsin, a divalent $F(ab')_2$ fragment and

destruction of the Fc will result. A divalent fragment is capable of causing agglutination.

Lysed (**choice B**) is not correct because it would require the integrity of the complement-binding regions of the IgM, which are found in the Fc, and the question stem does not provide complement in the mix.

Phagocytized (**choice C**) is not correct because it would require an intact cell-binding region in the Fc, and the question stem does not provide phagocytic cells in the mix.

Precipitated (**choice D**) would be the correct answer if the antigen in question were a soluble protein. Proteins precipitate when treated with specific antibodies, particles agglutinate. The two particles used in laboratory medicine are latex beads and erythrocytes. If neither of these is mentioned, then the student can assume that treatment would result in precipitation, not agglutination. Precipitation has exactly the same requirements as agglutination: a divalent antigen-binding molecule.

Unaffected (**choice E**) would be the correct answer if papain had been used to treat the isohemagglutinins. Because papain produces two monovalent Fab fragments, these are incapable of cross-linking antigen (whether soluble protein or particle), so neither agglutination nor precipitation would be possible.

27. **The correct answer is E.** The only way to identify a neonatal infection serologically is by detection of pathogen-specific IgM antibodies. This is because the fetus receives IgG antibodies from the mother by active transport across the placenta. Because you cannot identify the source of the antibodies, IgG detection in the child can simply reflect this natural passive type of protection. Because IgM does not cross the placenta, any IgM detected in the neonate is being produced in the child and is reflective of a response to infection. In this way, all children born to HIV-infected mothers will be seropositive by both ELISA and Western blot, but only 20% will actually be infected in utero, even in the absence of antiviral therapy.

    IgA (**choice A**) does not usually begin to be produced by a child until one to two years after birth. At the end of the first year, most children have no more than 20% of adult values, so it would not be a useful diagnostic in the neonate. Additionally, because *Toxoplasma gondii* is an intracellular parasite, IgA would not be the most effective immune response in any individual.

    IgD (**choice B**) will be produced by an infected neonate along with IgM because of alternative RNA splicing, but this is not a useful diagnostic. IgD rarely reaches levels easily detected by serology, and the immunoglobulin has the shortest half-life of all the immunoglobulins. The function of secreted IgD, if any, is not clear, so it is not a useful serologic test.

    IgE (**choice C**) does not usually begin to be produced by a child until well into the second year after birth. Additionally, because *Toxoplasma gondii* is an intracellular parasite, IgE would not be the most effective immune response in any individual.

    IgG (**choice D**) is not a useful serologic test in a neonate because it is impossible to determine the origin of such molecules. Children infected in utero will begin to produce IgG due to isotype switching late in gestation,

but because the placenta is actively transporting all maternal IgG into the fetus, it is not possible to distinguish whether the child is actually infected or simply passively protected using this technique.

28. **The correct answer is B.** IgM and secretory IgA are similar in that they are held together by a J chain synthesized by the B cell or plasma cell. Without the presence of the J chain, IgM would exist only in monomeric form, and the macroglobulin peak would be absent on electrophoresis. Because pentameric IgM is important for capturing newly introduced foreign antigen and thus beginning the immune response, the child is delayed in his development of protective responses to vaccination. Because secretory IgA is a dimer that protects the mucosal surfaces, such a child would be especially susceptible to infectious agents crossing the mucosal surfaces.

    Absence of CD40 (**choice A**) would affect the production of IgG, IgA, and IgE, but would not prevent macroglobulin synthesis. Indeed, most patients with this defect have hyper-macroglobulinemia because the CD40/CD40L interaction is necessary for isotype switching.

    Absence of IL-4 (**choice C**) would cause problems with the ability to produce IgG, IgA, and IgE. This cytokine, produced by Th2 cells, is necessary for the differentiation and development of most antibody responses other than IgM. Thus, IgM levels either would not be affected or would be increased in a compensatory fashion.

    Absence of Tdt (**choice D**) would cause problems with the patient's ability to perform the genetic rearrangements necessary to form the idiotype of the antibody molecule. They would not affect the isotype of antibody produced.

    Absence of Th2 cells (**choice E**) would affect the production of IgG, IgA, and IgE, but would not affect IgM production.

29. **The correct answer is B.** One of the most effective protective responses to infections with extracellular, encapsulated bacteria, such as *Streptococcus pneumoniae*, is complement-mediated opsonization. Because IgM is the most effective antibody at activating complement, generation of C3b fragments during this process coats the bacteria and makes them more susceptible to ingestion and intracellular killing by cells of the phagocytic system.

    ADCC (**choice A**), or antibody-dependent cell-mediated cytotoxicity, is a mechanism by which NK cells, neutrophils, macrophages, and eosinophils can use their Fc receptor to bind specific antibody and target an agent for lysis. No cells have Fc receptors for IgM, so this is not a mechanism that could act in concert with early IgM production.

    Cytotoxic T lymphocytes (**choice C**) identify altered-self/MHC class I molecule conjugates on the surfaces of cells that are malignantly transformed or infected with intracellular pathogens. They are not a protective mechanism that acts in concert with any antibody molecule.

    LAK cells (**choice D**), or lymphokine-activated killer cells, are NK cells that have been stimulated in vitro with cytokines that enhance their killing activity. These cells have a function in early surveillance against altered-self cells, but are not believed to play a role in protection against extracellular pathogens, such as this one.

NK cells (**choice E**) are members of the innate immune system and are believed to play a role in surveillance against tumor cells and other altered-self cells that fail to express MHC class I antigens on their surfaces. They would not act in concert with IgM production, and they would not be effective against an extracellular pathogen, such as this one.

30. **The correct answer is C.** The component of complement that is most important in clearance of extracellular pathogens such as *Streptococcus pneumoniae* is C3b. This fragment acts as an opsonin and enhances the ingestion and intracellular killing of the bacteria by phagocytic cells.

    C1 (**choice A**) is the first component of the complement cascade activated in the classic pathway. Although it is critical to initiating those events that can culminate in the production of the membrane attack complex, it is not the most important component for the clearance of infections such as this one.

    C2 (**choice B**) is the third component of the complement cascade activated in the classic pathway. Although it is critical to initiating those events that can culminate in the production of the membrane attack complex, it is not the most important component for the clearance of infections such as this one.

    C4 (**choice D**) is the second component of the complement cascade activated during the classic pathway. Although it is critical to initiating those events that can culminate in the production of the membrane attack complex, it is not the most important component for the clearance of infections such as this one.

    C5 (**choice E**) is the fifth component of the complement cascade activated during the classic pathway and the first step in the formation of the membrane attack complex (C5b–9). It is not the most important component for the clearance of infections such as this one.

31. **The correct answer is A.** The mucosal-associated lymphoid tissues (MALT) are the major sites of synthesis of IgA. IgA is a dimeric molecule held together by a J chain similar to that used in IgM. As IgA is transported across the epithelial surface, it acquires the secretory component, which functions both in transepithelial transport and protection from proteolytic cleavage.

    A monomeric immunoglobulin that crosses the placenta (**choice B**) describes IgG. IgG is the major immunoglobulin of the blood and is produced in lymph nodes and spleen, but less commonly in the MALT.

    A monomeric immunoglobulin bound by mast cells (**choice C**) describes IgE. IgE is the immunoglobulin that causes immediate hypersensitivity by virtue of its attraction to the Fc receptors of mast cells. It is not the major immunoglobulin produced in the MALT, although it may be produced there.

    A monomeric immunoglobulin that opsonizes (**choice D**) describes IgG. IgG is the major immunoglobulin of the blood and is produced in lymph nodes and spleen, but less commonly in the MALT.

    A pentameric immunoglobulin that activates complement (**choice E**) describes IgM. IgM is the major immunoglobulin of the primary immune response and is produced in lymph nodes and spleen, but less commonly in the MALT.

32. **The correct answer is D**. The transport of IgA dimers from the abluminal side of the mucosa to the lumen is mediated via attachment to polyimmunoglobulin receptors on mucosal cells. This allows endocytosis of IgA into the mucosal cell and secretion onto the other side. Secretory IgA found in the lumen of the bowel retains a residue of this receptor, secretory component, which further protects it from proteolytic cleavage inside the intestine. If this receptor were lacking, transport of IgA across the mucosa would not be possible, and the IgA dimers would be trapped on the abluminal side of the mucosa.

    Failure of isotype switching (**choice A**) is not a potential cause of such a condition because isotype switching occurs in secondary lymphoid organs and not in epithelial cells. Because the IgA dimers were present, isotype switching had been successful, but transepithelial transport was not occurring.

    Failure of variable domain gene segment rearrangement (**choice B**) is not a potential cause of such a condition because variable domain gene-segment rearrangement occurs in the primary lymphoid organs and not in epithelial cells. Because immunoglobulin was being produced, these gene segment rearrangements had occurred successfully, but transepithelial transport was not occurring.

    Loss of J chain synthesis (**choice C**) would result in the inability of an individual to join dimers of IgA and pentamers of IgM. Because the question states that the individual was making IgA dimers, J chain is clearly being made successfully by the B cell.

    Loss of Th2 cells (**choice E**) would cause the patient to be unable to switch isotypes. These persons could make only IgM, and this patient clearly has successfully produced IgA.

33. **The correct answer is A.** The killer cells cytotoxic to targets lacking MHC class I antigens are NK cells. These cells are members of the innate immune response, and as such their response is not enhanced over time. The most specific, inducible cytotoxic cells in the body are cytotoxic T lymphocytes (CTLs), which depend on MHC class I recognition of their target. Because this question asks how the tumor cells can be altered to make them better stimulators of an immune response, one approach would be to increase their expression of MHC class I molecules. This can be accomplished by treatment of the tumor cells with interferon (IFN)-γ. IFN-γ increases expression of both class I and II MHC products on cells.

    IL-2 (**choice B**) is a product of Th1 lymphocytes and induces proliferation of antigen-primed Th and cytotoxic T cells. It also supports their long-term growth. It would not have an effect on this patient's tumor cells.

    IL-8 (**choice C**) is a product of macrophages and endothelial cells and acts on neutrophils to cause their chemotaxis and extravasation into tissues. It would not have an effect on this patient's tumor cells.

    IL-10 (**choice D**) is a product of Th2 cells and acts on macrophages to suppress their cytokine production. It therefore indirectly reduces cytokine production by Th1 cells and dampens the activation of the cell-mediated arm of the immune response. It would not have an effect on this patient's tumor cells.

    TNF-β (**choice E**) is a product of macrophages and NK cells and acts on tumor cells to cause direct cytotoxicity. It acts on inflammatory cells to

induce cytokine secretion and causes the cachexia associated with chronic inflammation. It would not cause the patient's tumor cells to stimulate better immunity.

34. **The correct answer is D.** CD8+ lymphocytes, or cytotoxic T lymphocytes recognize their target cells by binding to MHC class I molecules containing altered-self peptides. The class I molecule is a two-chain structure, with one long α chain that passes through the cellular membrane and a shorter chain called β_2 microglobulin that becomes associated with the α chain.

    A β_2 domain instead of a β_2 microglobulin (**choice A**) describes the class II MHC molecule. It is loaded with peptides by the endosomal (exogenous) pathway and is recognized by CD4+ T cells.

    Invariant chains (**choice B**) are found blocking the peptide-binding groove of the class II MHC molecule immediately after synthesis. These chains are digested away when the class II MHC is exposed to the contents of the phagocytic vesicles of macrophages, and the groove is loaded with peptides from the ingested particle.

    A peptide-binding groove (**choice C**) would be found in both class I and II MHC molecules and is therefore not the best answer.

    Two similar chains (**choice E**) would be found in the class II MHC molecule. It is composed of an α and a β chain of similar lengths, both of which have transmembrane domains. The class II MHC molecule is loaded with peptides by the endosomal (exogenous) pathway and is recognized by CD4+ T cells.

35. **The correct answer is A.** Although the ablation of the thymus in early childhood will ultimately have far-reaching consequences in the development of many immune responses, the immune surveillance of tumors is performed only by cytotoxic T cells and NK cells, and thus would be profoundly affected by this treatment. Although NK cell numbers would not be affected by loss of the thymus, in the absence of Th1 cell cytokines, they would not be able to increase in number in response to challenge. Other parameters that could be depressed include immune responses to intracellular pathogens and secondary antibody responses.

    Depressed oxygen-dependent killing by neutrophils (**choice B**) would not be expected in this case because neutrophils are components of the innate immune response and function in the absence of T-cell help.

    Depressed primary response to soluble antigens (**choice C**) would not be expected in this case because the IgM response to many antigens is T-cell independent. It is class switching that would be impossible without T-cell help.

    Increased cellularity of lymph node paracortical areas (**choice D**) would not be expected in this case because the paracortex of lymph nodes is a T-cell area. Therefore, following thymic irradiation, decreased cellularity of these regions would occur.

    Increased tendency toward atopy (**choice E**) would not be expected in this case because atopic allergies are those that involve IgE antibodies and mast cells. IgE cannot be produced without T-cell help, so athymic individuals will have decreased tendency toward atopy.

36. **The correct answer is A.** The Mantoux test, or tuberculin test (or simply the TB skin test), is the classic clinical demonstration of the function of the delayed-type hypersensitivity response. This is a cell-mediated reaction caused by sensitization of Th1 cells and demonstrated by the influx and activation of macrophages in response to the cytokines that they elaborate.

    That a humoral immune response has occurred (**choice B**) is not true. Antibodies are not involved in the production of a DTH response, and they are not important products during infections with most intracellular pathogens.

    That the B-cell system is functional (**choice C**) is not true. B cells do not play a role in the DTH response, and they do not play a major role in defense during infections with most intracellular pathogens.

    That the B- and T-cell systems are functional (**choice D**) is not true. The DTH response certainly demonstrates that the Th1 response is functional, but it says nothing about the function of B cells.

    That the neutrophilic phagocyte system is functional (**choice E**) is not true. Neutrophils do not play a role in the elicitation of the DTH response. Neutrophils are the important cells in abscess formation, not granuloma formation.

37. **The correct answer is D.** The goal of this therapy is to provide an increased population of activated antigen-presenting cells primed with tumor cell antigens so that these can be presented to the Th cells involved in stimulation of cell-mediated immunity. The Th1 cell is the first cell listed which would be activated by such a treatment.

    B lymphocytes (**choice A**) would not be stimulated by such treatment. B lymphocytes bind to and are activated by unprocessed (not cell-bound) antigens.

    Cytotoxic T lymphocytes (**choice B**) would be indirectly stimulated by this treatment. Cytokines secreted by the activated Th1 cells would have the effect of increasing the number and cytotoxic activity of killer cells.

    NK cells (**choice C**) would be indirectly stimulated by this treatment. Cytokines secreted by the activated Th1 cells would have the effect of increasing the number and cytotoxic activity of killer cells.

    Th2 cells (**choice E**) would be stimulated by this treatment, but this is not the major goal of such therapy. Th2 cells stimulate humoral immunity, which is not the most important protective mechanism against tumor cells.

38. **The correct answer is D**. The protective response to the tetanus toxoid depends on production of antibodies that prevent the binding of the toxin. After repeated immunizations, the population of memory B cells is stimulated, which is the goal of such prophylaxis. Memory B cells may have IgG, IgA, or occasionally IgE on their surfaces serving as antigen receptors.

    That their receptors would have high avidity (**choice A**) is not true because avidity decreases with repeated booster inoculations. This is because IgM, which is the immunoglobulin of the primary immune response and is the receptor on mature naive B lymphocytes, is replaced in secondary and subsequent responses by isotype switching to other isotypes such as IgG or IgA or IgE. All of these molecules have less avidity than IgM because they have

fewer combining sites than IgM. The secondary and subsequent responses should have increased affinity (goodness-of-fit of idiotype for epitope), but decreased avidity.

That they would be large and highly metabolic (**choice B**) is not true because memory lymphocytes are usually small and in a resting phase of the cell cycle. Activated lymphocytes are large and highly metabolic.

That they would have low levels of adhesion molecules (**choice C**) is not true because memory lymphocytes express high levels of adhesion molecules. This allows them to migrate to areas of active inflammation where they can have maximum benefit in protection of the host.

That they would have surface IgM (**choice E**) is not true because this would describe mature, naive B lymphocytes that have not met their antigen before. As soon as the primary response begins, isotype switching to other classes of immunoglobulin is directed by Th cells.

39. **The correct answer is A.** Because rabies antitoxin is a pooled, human immunoglobulin product, repeated inoculation will cause a patient to produce anti-allotype antibodies. Allotypes are minor amino-acid sequence variations in the constant domains of heavy and light immunoglobulin chains. Their expression is genetically determined, and repeated exposure to molecules of foreign allotype can cause antibodies to be produced which recognize these sequence variations.

    Anti-epitope antibodies (**choice B**) would be produced by repeated inoculation of an immunogen. The epitope of the antigen has a three-dimensional complementarity with the idiotype of the antibody molecule. In this case, anti-epitope antibodies would be generated by rabies vaccination, but the question asks what the result of repeated exposure to immunoglobulins would be.

    Anti-idiotype antibodies (**choice C**) would be generated in a human if a monoclonal antibody preparation were repeatedly inoculated. The idiotype of an antibody is the three-dimensional shape of its antigen-combining site. It is unique to the antibodies produced by a clone of cells. Because the material mentioned in this case is a pooled human immunoglobulin, it would contain many different idiotypes and would be unlikely to elicit any one specific anti-idiotype antibody.

    Anti-isotype antibodies (**choice D**) are usually raised across species barriers. For example, to produce anti-human IgG, IgG pooled from many humans is repeatedly injected into rabbits, goats, or sheep. These animals will recognize the human determinants in the constant domains of the heavy and light chains (the isotypes) and will produce antibodies that specifically recognize those determinants.

    Anti-rabies antibodies (**choice E**) are generated during vaccination. When the killed virus is administered, the patient makes an active, artificial response to the immunogen and produces immunoglobulins, which will protect against virus attachment. In this case, anti-rabies antibodies were inoculated, so there is no possibility that more of the same will be generated.

40. **The correct answer is B.** In this case, high-risk individuals are vaccinated with the serotype of influenza virus that is predicted to be most common in this flu season. This elicits an active immunologic response in the patient

and is artificial by definition because it is being administered in a medical setting. This sort of immunization causes the development of memory in the patient that will protect for the whole season, but it requires approximately two weeks for development of protection.

Adaptive (**choice A**) immunity describes all immune responses that have specificity and memory. These immune responses are produced by specific B and T lymphocytes. Although adaptive immunity will be elicited in these patients, this is not the best answer because it is imprecise.

Artificial passive (**choice C**) immunity is achieved when preformed immunologic products (immune cells or antibodies) are given to a patient. These procedures provide passive protection that is rapid but lacks immunologic memory. Because it is administered in a medical setting, it is, by definition, artificial.

Natural active (**choice D**) immunity would result following recovery from an infection.

Natural passive (**choice E**) immunity is acquired across the placenta and in the colostrum and breast milk, from mother to child. The child receives preformed antibodies (IgG across the placenta and IgA in milk) that protect the child until a natural active immune response can be mounted.

41. **The correct answer is C.** In this case, an attempt at postexposure prophylaxis against tetanus is made by inoculating antitetanus immunoglobulin into the patient. When preformed immunologic products (immune cells or antibodies) are given to a patient, the procedure provides passive protection that is rapid but lacks immunologic memory. Because it is being administered in a medical setting, it is by definition artificial.

    Adaptive (**choice A**) immunity describes all immune responses that have specificity and memory. These immune responses are produced by specific B and T lymphocytes. Because this patient is being given a product of the adaptive immune response (antibodies), there will be no elicitation of an adaptive immune response in this individual.

    Artificial active (**choice B**) immunity is produced during the process of vaccination. The patient is exposed to a modified pathogen or product. As a result, an active immune response to that inoculation is made. This sort of immunization causes the development of memory in the patient.

    Natural active (**choice D**) immunity would result after a recovery from an infection.

    Natural passive (**choice E**) immunity is acquired across the placenta and in the colostrum and breast milk, from mother to child. The child receives preformed antibodies (IgG across the placenta and IgA in milk), which serve to protect the child until a natural active immune response can be mounted.

42. **The correct answer is A.** Allotypes are minor amino-acid sequence variations in the constant domains of heavy and light immunoglobulin chains. Their expression is genetically determined, and variations can be used as evidence in favor of paternity in some cases. Allotypic markers are most frequently used in studies of population genetics, as certain ethnic groups are likely to have similar allotypic markers on their immunoglobulins.

Allotypic markers do not affect the biologic function of the immunoglobulin molecule.

The term "idiotype" (**choice B**) describes the 3-dimensional shape of the antigen-combining site of an antibody or T-cell receptor molecule. Because each human is capable of producing many millions of different idiotypic sequences, these would not be useful in paternity cases.

IgA2 (**choice C**) is an isotype of immunoglobulin. Because all normal human beings produce some amount of this immunoglobulin, it would not be useful in paternity cases.

IgM (**choice D**) is an isotype of immunoglobulin. Because all normal human beings produce some amount of this immunoglobulin, it would not be useful in paternity cases.

An isotype (**choice E**) is found in the heavy- or light-chain constant domains of an immunoglobulin. Thus, there are 5 heavy-chain isotypes (A, E, G, M, and D) and two light-chain isotypes (κ and λ). Because all human beings produce heavy- and light-chain isotypes, this would not be useful in paternity testing.

43. **The correct answer is D.** Although this vaccine is no longer in use because of the possible side effects of *Bordetella pertussis* inoculation, in this case the whole, killed bacteria served as an adjuvant. They increased local inflammation, thus calling inflammatory cells to the site and prolonging exposure to the immunogen, the capsular polysaccharide of *Haemophilus*.

    A carrier (**choice A**) is not correct because a carrier is a protein covalently coupled to a hapten to elicit a response. There is no mention in the question stem here that the polysaccharide is chemically coupled to the bacteria; it is stated that they are only mixed together.

    A hapten (**choice B**) is not correct because a hapten is a single antigenic epitope, and a whole, killed bacterium such as *Bordetella* has many epitopes.

    A mitogen (**choice C**) is not correct because mitogens are substances that cause the polyclonal activation of immune cells. The mitogens most commonly used in clinical laboratory medicine are lipopolysaccharide, concanavalin A, and pokeweed mitogen.

    An immunogen (**choice E**) is not correct because the immunogen in a vaccine is the substance to which the immune response is being made. Because the object of the Hib vaccine is to immunize against *Haemophilus influenzae, Bordetella pertussis* bacteria cannot be the immunogen.

44. **The correct answer is D.** This is a case of DiGeorge syndrome, which is a congenital failure in the formation of the third and fourth pharyngeal pouches. As a result, individuals with this defect have aplastic thymus and parathyroids and facial, esophageal, and cardiac malformations. Immunologically, the absence of the thymus will ultimately have global effects on the development of all T-cell–mediated immune responses. At birth, the child will have IgG antibodies that have been transplacentally transferred from the mother, but by 9 months or so after birth, these will be gone and IgM will be the only isotype of immunoglobulin present. Phagocytic killing will be normal until that point, although after all the maternal IgG is gone, opsonization of bacteria will no longer be possible.

Antibody-dependent cell-mediated cytotoxicity of parasite targets (**choice A**) will be depressed in this child because eosinophil-mediated ADCC requires IgE antibodies, and these cannot be produced without T-cell help.

Cellularity of splenic periarteriolar lymphoid sheaths (**choice B**) will be decreased in this child because these are T-cell–dependent areas of the spleen.

Cytotoxic killing of virus-infected targets (**choice C**) will be depressed in this child because cytotoxic T cells will be absent, and only NK cells will be available for antiviral protection.

The proliferative response to concanavalin A (**choice E**) will be depressed in this child because concanavalin A is a T-cell mitogen. If there are no T cells, there will be no proliferation in response to this mitogen.

45. **The correct answer is A.** This is a case of X-linked agammaglobulinemia, or Bruton agammaglobulinemia. It is caused by a mutation in a tyrosine kinase gene, which is important in B-cell maturation. The bone marrow becomes hypercellular with cells that cannot progress beyond the pre-B stage, while the peripheral blood lacks mature B lymphocytes. There will be no proliferative response to B-cell mitogens (pokeweed mitogen), and CD19+ cells will be absent from the blood. Persons with this condition are unable to mount a normal antibody response; therefore, symptoms appear after the disappearance of maternal antibodies. Susceptibility to extracellular, encapsulated pathogens is profound.

Common variable hypogammaglobulinemia (**choice B**) is a condition that usually appears in the late teens or early twenties. It is believed to be an autoimmune disease and is associated with the disappearance of immunoglobulin isotypes over time.

DiGeorge syndrome (**choice C**), or congenital thymic aplasia, is a condition in which there is failure of formation of the third and fourth pharyngeal pouches. These infants have facial abnormalities, failure of formation of the parathyroids, and cardiac defects, as well as absence of T-lymphocyte development.

Selective immunoglobulin deficiency (**choice D**) would not be manifested by a failure of B-cell development in the bone marrow. Selective IgA deficiency is most common of these and would manifest as increased susceptibility to mucosal-surface pathogens.

Wiskott-Aldrich syndrome (**choice E**) is a complex immune deficiency with a triad of symptoms: eczema, thrombocytopenia, and immunodeficiency. It is inherited in an X-linked recessive fashion. These patients are prone to development of malignant lymphomas and have inability to respond to polysaccharide antigens.

46. **The correct answer is B.** Unusual frequency or severity of *Neisseria* infections should always lead to a suspicion of a terminal complement component deficiency (C5, C6, C7, or C8). *Neisseria* seems to be highly susceptible to complement-mediated lysis, so any failure of production of the membrane attack complex predisposes the patient to recurrent bacteremias with these organisms.

Common variable immunodeficiency (**choice A**) is a condition that usually appears in the late teens or early twenties. It is believed to be an

autoimmune disease and is associated with the disappearance of immunoglobulin isotypes over time.

DiGeorge syndrome (**choice C**) is a condition in which there is failure of formation of the third and fourth pharyngeal pouches. Diagnosed in infancy, these individuals have facial abnormalities, failure of formation of the parathyroids, and cardiac defects, as well as an absence of T-lymphocyte development. This condition predisposes to early viral and fungal infections.

Selective IgA deficiency (**choice D**) would be expected to result in respiratory and gastrointestinal tract infections, autoimmune disease, and allergies.

Severe combined immunodeficiency (**choice E**) typically presents with early susceptibility to viral and fungal agents. It is most frequently diagnosed in infancy, after the disappearance of maternally derived IgG antibodies.

47. **The correct answer is E.** The description of painful abdominal edema and edema in the oral mucosa are typical of hereditary angioedema. This is a genetic deficiency of complement C1 inhibitor. When this important control protein is missing, there is excessive use of the classic complement pathway components, especially C4. This causes abnormal inflammation along the mucosal surfaces.

    Abnormal superoxide anion production by neutrophils (**choice A**) would result in predisposition to infections with extracellular pathogens.

    Abnormal T-cell function (**choice B**) would result in predisposition to infections with viral and fungal pathogens, not edema of the mucosal surfaces.

    Abnormal T-cell numbers (**choice C**) would result in predisposition to infections with viral and fungal pathogens, not edema of the mucosal surfaces.

    Defective neutrophil chemotaxis (**choice D**) would result in neutrophilia and failure to produce pus and abscesses in response to extracellular bacterial invasion.

48. **The correct answer is D.** The infections of this child with catalase-positive bacteria are characteristic of chronic granulomatous disease (CGD). While two thirds of CGD patients are male, one third has the autosomal recessive form of NADPH oxidase deficiency and can be female.

    Adenosine deaminase deficiency (**choice A**) produces a severe combined immunodeficiency. The infections seen are likely to be the result of T-cell deficiency (viral and fungal agents). In the absence of adenosine deaminase, deoxyadenosine phosphate builds up in T cells and is toxic to them.

    C1 inhibitor (**choice B**) is not an enzyme, and its absence does not predispose to infections. It is absent in the condition known as hereditary angioedema, represented by recurrent, painful bouts of mucosal edema.

    Myeloperoxidase (**choice C**) deficiency is normally without clinical symptoms. This is an enzyme that is important in intracellular killing in phagocytes because it causes formation of toxic halide radicals. However, because oxygen radicals are more important in intracellular killing, MPO deficiency will present without symptoms.

    Superoxide dismutase (**choice E**) deficiency has not been described in leukocytes, and its absence would not be likely to predispose to infection.

49. **The correct answer is B.** This is a case of X-linked agammaglobulinemia or Bruton agammaglobulinemia. During the early 1990s, the gene responsible for this condition was cloned. The normal counterpart of the mutant gene encodes a protein tyrosine kinase (Bruton tyrosine kinase, *Btk*), which is important in B-cell signaling. When it is absent or altered, B lymphocytes are unable to progress beyond the pre-B cell stage in the bone marrow. Thus, the bone marrow becomes hypercellular, while the peripheral blood is lacking mature B lymphocytes. Persons with this condition are unable to mount a normal antibody response; therefore, symptoms appear after the disappearance of maternal antibodies, and susceptibility to extracellular, encapsulated pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* is profound.

    Adenosine deaminase deficiency (**choice A**) is an example of a severe combined immunodeficiency disease (SCID). When this enzyme is absent, toxic metabolites build up in B and T lymphocytes and cause a general failure of the immune response. It would have clinical manifestations of both B- and T-lymphocyte defects, and not exclusively B lymphocytes, as described in this case history.

    A defect of the *SAP* gene (**choice C**) is believed to cause X-linked proliferative disease, in which uncontrolled T-cell proliferation follows infection with Epstein-Barr virus. *SAP* stands for SLAM-associated protein, and SLAM (signaling lymphocytic activation molecule) is a potent T-cell coactivator.

    Defect of the *WAS* gene (**choice D**) causes Wiskott-Aldrich syndrome, in which a defect in CD43 (a cytoskeletal protein) causes defects in T cells and platelets. Patients with Wiskott-Aldrich syndrome display a triad of signs: thrombocytopenia, eczema, and immunodeficiency.

    ICAM-1 deficiency (**choice E**) would cause defects of antigen recognition and activation of lymphocytes. ICAM-1 is an adhesion molecule in the immunoglobulin superfamily of genes and is bound by LFA-1 integrin.

50. **The correct answer is B.** This is a case of hereditary angioedema, caused by a deficiency in an important complement regulatory protein, C1-INH. When it is absent, the early components of the classical complement cascade are overused. It is normally diagnosed by the finding of depressed levels of complement component C4 in the blood.

    Depressed C3 (**choice A**) would not be a correlate of C1-INH deficiency. There are separate regulatory controls on abnormal complement activation that operate at the C3 level, so this condition is rarely found.

    Depressed C5 (**choice C**) would not be a correlate of C1-INH deficiency. There are separate regulatory controls on abnormal complement activation that operate at the C5 level, so this condition is rarely found.

    Elevated C1 (**choice D**) would not be found in this case because the condition results in the overuse of early components of the classical complement cascade. Therefore, serum levels of C1, C4, and C2 would be decreased from normal values.

    Elevated C1, C4, and C2 (**choice E**) would not be found in this case because the condition results in the overuse of early components of the classical complement cascade. Therefore, serum levels of C1, C4, and C2 would be decreased from normal values.

51. **The correct answer is B**. This child has bare lymphocyte syndrome, a rare autosomal-recessive disease in which there is absence of MHC class II molecules on cells. Thus, her cells can recognize other cells as foreign and proliferate to T-cell mitogens, but they cannot be recognized by allogeneic lymphocytes because they do not express class II MHC antigens on their surface. The phrase which best describes the MHC class II molecule on this list is that it is designed to bind exogenously processed peptides. Other descriptions that could apply would be that it has two chains of similar length, is produced with an invariant chain, and is designed to present foreign peptides to Th cells.

    It is designed to bind endogenously produced peptides (**choice A**) is a description that fits the class I MHC molecule. If this were a case of class I MHC deficiency, she would not have made a normal proliferative response to mismatched allogeneic cells.

    It possesses β_2 microglobulin (**choice C**) is a description that fits the class I MHC molecule.

    It possesses two chains of unequal length (**choice D**) is a description of the class I MHC molecule. It has an α chain with 3 domains, and a smaller chain, β_2 microglobulin, becomes associated with the α chain.

    It should be present on all nucleated cells in the body (**choice E**) describes the class I MHC molecule. Class II MHC will be found on all antigen-presenting cells in the body.

52. **The correct answer is E.** IL-10 is produced by Th2 cells and inhibits Th1 cells. Because the response to poison ivy is a delayed-type hypersensitivity response and therefore is mediated by Th1 cells and macrophages, inhibiting their activity would minimize the severity of the reaction.

    γ-Interferon (**choice A**) is a product of Th1 cells, CTLs, and NK cells. It inhibits the proliferation of Th2 cells and therefore would skew the immune response toward a more potent cell-mediated arm. This is not a cytokine that would help this patient: It would make his condition worse.

    IL-2 (**choice B**) is a product of Th cells that induces the proliferation and enhances the activity of antigen-primed Th cells and CTLs. It would tend to increase the symptoms of this patient, not ameliorate them.

    IL-3 (**choice C**) is a product of Th cells and NK cells. It acts on hematopoietic cells to encourage myeloid cell development. It would neither hinder nor help this man's condition.

    IL-8 (**choice D**) is a product of macrophages and endothelial cells and acts on neutrophils to attract them to areas of inflammation. It would increase inflammation in the area.

53. **The correct answer is A**. The condition described here is an immune complex–mediated pathology. When large amounts of antigen are added into a situation where there is pre-existence of a large amount of antibody, the precipitation of those complexes in the small vasculature causes a type III hypersensitivity response. The only syndrome on this list that also has a type III etiology is the Arthus reaction.

    Atopic allergy (**choice B**) is a type I hypersensitivity, mediated by IgE antibodies and mast cells. Although many parasitic diseases elicit IgE and

ADCC by eosinophils, the question stem clearly stipulates the presence of IgG antibodies, so a type I hypersensitivity reaction is ruled out.

Goodpasture syndrome (**choice C**) and transfusion reaction (**choice E**) are examples of a type II, cytotoxic antibody hypersensitivity response. In these cases, antibody binds to cells or tissues of the body and elicits complement activation in those locations. The result is a tissue- or organ-specific pathology, and not a systemic problem as described here.

The tuberculin reaction (**choice D**) is a type IV hypersensitivity response. Delayed-type hypersensitivities are mediated by Th1 and Th17 cells and their mediators and have no contribution whatsoever from antibodies.

54. **The correct answer is E.** The description in the vignette is a type I hypersensitivity reaction—the only type of hypersensitivity that can be manifested in minutes. These are IgE-mediated responses and are an important protective response against helminth parasites that migrate through the tissues.

    The Arthus reaction (**choice A**) is an example of a type III (immune complex–mediated) pathology. These hypersensitivities develop when antigen is added to pre-existing antibody and immune complexes are filtered out of the circulation in the small vasculature. Complement is activated in these locations, and the underlying tissue is damaged. In this vignette, the killing is occurring in the skin and is associated within minutes with stinging, itching, and urticaria.

    Contact dermatitis (**choice B**) is an example of a type IV (delayed-type) hypersensitivity response. After the sensitizing exposure, symptoms of this type of hypersensitivity will occur in 48 to 72 hours (not minutes, as described here).

    Passive cutaneous anaphylaxis (**choice C**) is an example of a type I hypersensitivity reaction, but it is used to diagnose these conditions using passive transfer of serum. It is not a mechanism of protection, but a diagnostic technique.

    Serum sickness (**choice D**) is an example of a type III (immune complex-mediated) pathology. These hypersensitivities develop when antigen is added to pre-existing antibody and immune complexes are filtered out of the circulation in the small vasculature. Complement is activated in these locations, and the underlying tissue is damaged. In this vignette, the killing is occurring in the skin and is associated within minutes with stinging, itching, and urticaria.

55. **The correct answer is D.** An isograft is performed between genetically identical individuals. In human medicine, these are performed between monozygotic twins. In reality, even these "identical" individuals are not identical because minor mutations can occur during development. These sorts of grafts still require immunosuppression for success. They are, however, the best chance for success other than autografts.

    An allograft (**choice A**) is a transplant between two members of the same species who are not genetically identical. These are the most common types of transplants used in medicine, but in this vignette, the twins are described as having identical MHC haplotypes.

An autograft (**choice B**) is a transplant from one location in the body to another. This is the only form of transplantation that will succeed without immunosuppression.

"Heterograft" (**choice C**) is not a word that is used in transplantation immunology.

A xenograft (**choice E**) is a transplant that is performed across species barriers.

56. **The correct answer is C.** Graft-versus-host disease (GVHD) is primarily a manifestation of sensitization of transplanted T cells against recipient tissues. The killing of mucosal and other epithelial cells is largely mediated through recognition of MHC class I incompatibility by transferred cytotoxic cells or their precursors. However, eventually, continuous priming by the host's own tissues will elicit immune responses at the level of all the cells of the immune system.

    Activated macrophages (**choice A**) are involved in the delayed-type hypersensitivity response, but are not stimulated by MHC incompatibility, so if they become involved in pathology, it has to be secondary to Th stimulation.

    Antibodies and complement (**choice B**) are not involved in GVHD. Because bone marrow is a cellular transplant, it is the cells inside it that start the problem, not accidentally transferred antibodies or complement.

    LAK (lymphokine-activated killer) cells (**choice D**) are believed to be involved in the rejection of bone marrow transplants by the recipient (host-versus-graft), but not in GVHD.

    NK cells (**choice E**) are believed to be involved in the rejection of bone marrow transplants by the recipient (host-versus-graft), but not in GVHD.

57. **The correct answer is C.** The lowest stimulation index (and the lowest amount of proliferation) is shown between sibling 1 and the prospective recipient, both when the donor cells are used as stimulators and as responders. This means (most importantly) that the recipient will make little response to the graft and (less importantly, except in graft-versus-host disease) that the donor will make little response against the recipient.

    The father (**choice A**) is not the best choice of donors because the recipient makes 4 times the proliferative response to his cells as to those of sibling 1.

    The mother (**choice B**) is not the best choice of donors because the recipient makes twice the proliferative response to her cells as to those of sibling 1. She would be the second-best choice, unless sibling 1 had an incompatible ABO blood group.

    Sibling 2 (**choice D**) is not the best choice of donors because the recipient makes 8 times the proliferative response to his cells as to those of sibling 1.

    Sibling 3 (**choice E**) is not the best choice of donors because the recipient makes 8 times the proliferative response to his cells as to those of sibling 1.

58. **The correct answer is A.** IFNs of all types increase cellular expression of MHC class I and II products. Because CD8+ cells recognize their targets by MHC class I–dependent mechanisms, increases in the amount of these antigens on tumor-cell targets would increase susceptibility to cytotoxic killing.

IL-1 (**choice B**) does not increase MHC class I molecule expression. The endogenous pyrogen is responsible for alteration of the hypothalamic temperature set point during acute inflammatory events.

IL-2 (**choice C**) does not increase MHC class I molecule expression. It is produced by Th cells and causes proliferation of many classes of lymphocytes.

IL-10 (**choice D**) does not increase MHC class I molecule expression. It is a product of Th2 cells and inhibits Th1 cells; thus, it inhibits the cell-mediated arm of the immune response.

TNF-α (**choice E**) does not increase MHC class I molecule expression. It may act directly on tumor cells to cause their necrosis and decrease angiogenesis. It is a product of Th1 cells that stimulates the effector cells of cell-mediated immunity.

59. **The correct answer is D.** The standard screening test for HIV infection is the enzyme-linked immunosorbent assay, or ELISA. In this test, the virus p24 antigen is coated onto microtiter plates. Serum from the test subjects is added, followed by antihuman-immunoglobulin, which is labeled with an enzyme. When the substrate for the enzyme is added, if the antibodies listed have bound in sequence, there will be a color change in that microtiter well.

Electrophoresis of HIV antigens in polyacrylamide gel (**choice A**) describes the Western blot, which is used as a confirmatory test of HIV infection.

HIV antigen covalently coupled to RBC, patient serum, and anti-immunoglobulin (**choice B**) describes an erythrocyte agglutination test. There is no such test in use for diagnosis of HIV. The indirect Coombs test, which is used to detect Rh– mothers who have become sensitized to the Rh antigens of their fetuses, operates on this principle, however.

HIV antigen covalently coupled to RBC, patient serum, and complement (**choice C**) describes either a complement-fixation or complement-mediated hemolysis assay. There is no such test in use for the diagnosis of HIV.

HIV antigen, patient serum, anti-immunoglobulin serum, and radioactive ligand (**choice E**) describes a radioimmunoassay. This is not used in the standard screening for HIV.

60. **The correct answer is D.** If the child is developing hemolytic disease of the newborn, then his erythrocytes will already be coated with maternal anti-Rh antibodies. Adding Coombs serum (antihuman gammaglobulin) to the baby's RBCs then will cause agglutination. This is the direct Coombs test.

Mother's serum plus RhoGAM plus Coombs reagent (**choice A**) is not a set of reagents that will accomplish any diagnosis. RhoGAM is anti-RhD immunoglobulin, which is given to Rh– mothers at the termination of any Rh+ pregnancy. If the mother is sensitized, she is making IgG antibodies of the same specificity. Adding these 3 reagents together would tell you nothing of the baby's condition.

Mother's serum plus Rh– RBCs plus Coombs reagent (**choice B**) is not a set of reagents that will accomplish any diagnosis. If the mother is Rh–, she will not make a response to Rh– RBCs, and addition of Coombs reagent will accomplish nothing.

RhoGAM plus Rh+ RBCs from the baby (**choice C**) is not a set of reagents that will accomplish any diagnosis. RhoGAM will bind to Rh+ RBCs from the baby by definition, but adding these reagents together would tell you nothing of the baby's condition.

Rh+ RBCs plus mother's serum plus Coombs (**choice E**) is the set of reagents necessary for the performance of the indirect Coombs test. This is a test used to determine if the mother is making IgG anti-Rh antibodies, which could cross the placenta and harm a fetus. The question asks about the direct Coombs test, not the indirect.

61. **The correct answer is D.** The cell surface marker that would be useful to identify NK cells is CD56. The cells that have the highest fluorescence with antibodies to CD56 are found in quadrant D of panel 1.

    Panel 1, quadrant A (**choice A**) contains the cells with maximum fluorescence with antibodies to CD3. These would be T lymphocytes.

    Panel 1, quadrant B (**choice B**) contains the cells double-labeled with CD3 and CD56. Because CD3 is the pan-T-cell marker and CD56 is an NK-cell marker, there are no double-labeled cells in this case.

    Panel 1, quadrant C (**choice C**) contains the cells that have background fluorescence with both CD3 and CD56. These are non-T, non-NK cells, so they could be B lymphocytes or any other leukocyte.

    Panel 2, quadrant A (**choice E**) contains the cells with maximum fluorescence with antibodies to CD3. These are T lymphocytes.

    Panel 2, quadrant B (**choice F**) contains double-labeled cells, which fluoresce with both antibody to CD3 and antibody to CD20. Because CD3 is a T-cell marker and CD20 is a B-cell marker, there are no cells in this quadrant.

    Panel 2, quadrant C (**choice G**) contains the cells with background fluorescence with both antibodies to CD3 and CD20. These would be non-B, non-T cells and would contain some NK cells, but other leukocytes would be included here, so this is not the best choice.

    Panel 2, quadrant D (**choice H**) contains the cells with maximum fluorescence with antibody to CD20, which is a B-cell marker.

62. **The correct answer is D.** Coombs reagent is antihuman IgG. It is necessary in the direct and indirect Coombs tests because in those cases, one is looking for IgG antibodies that could be transported across the placenta to harm an unborn child. IgG is a much smaller molecule than IgM, and is not capable of agglutinating erythrocytes without the addition of a "developing" antibody. In the ABO blood typing test, the important isohemagglutinins are of the IgM variety, capable of agglutinating erythrocytes by themselves because of their sheer size.

    The statement that all antibodies made in response to blood glycoproteins are IgG (**choice A**) is not true because isohemagglutinins against ABO blood group antigens are IgM.

    The statement that complement-mediated lysis is not important in ABO incompatibilities (**choice B**) is not true because isohemagglutinins of the IgM variety are extremely powerful activators of complement-mediated

lysis. The agglutination tests here, however, do not use complement-mediated lysis as the indicator system.

That Coombs serum identifies only anti-Rh antibodies (**choice C**) is not true. Coombs serum is antihuman IgG. It will bind to the Fc portion of any human IgG molecule, regardless of its antigenic specificity.

The statement that the high titer of natural isohemagglutinins makes Coombs reagent unnecessary (**choice E**) is not true. It is the isotype of these antibodies (IgM) and the size of that molecule that allows agglutination to proceed without a developing antibody.

63. **The correct answer is A.** The direct fluorescent antibody test is used to detect antigens in the tissues of a patient.

The enzyme-linked immunosorbent assay (**choice B**) is a test usually used to detect antibody production. It can be modified to detect antigen, but not from a tissue specimen such as this one.

The indirect fluorescent antibody test (**choice C**) is used to detect antibodies being produced in a patient. It is not used for detection of microbial antigens.

Radioimmunoassay (**choice D**) is generally used to detect antibodies in a patient.

Western blot (**choice E**) is used to detect antibodies in a patient, not antigen.

64. **The correct answer is D.** To generate tumor-specific killer cells in vitro that would kill tumor cells transfected with an altered-self MHC class I gene, one would need to start with potential killer cells that use MHC I as a stimulatory signal. The only cytotoxic cell in the body that meets these criteria is the cytotoxic T lymphocyte (CTL). In panel I, increasing levels of fluorescence with antibody to CD8 are plotted as one moves to the right, and increasing levels of fluorescence with antibody to CD4 are plotted as one moves upward. Thus, the cells most strongly positive with CD8 are found the farthest to the right in quadrant D of panel I.

Panel I, quadrant A (**choice A**) would contain cells that are CD4+ and CD8–. These would be helper cells, and they would not be cytotoxic to transfected tumor cells.

Panel I, quadrant B (**choice B**) would contain cells that are double-positive for CD4 and CD8. These cells would be found as immature thymocytes in the thymus and not in the blood; thus, there are no double-labeled cells shown in this quadrant.

Panel I, quadrant C (**choice C**) contains the cells that have only background levels of fluorescence with antibodies to CD4 and CD8. These would be nonhelper, noncytotoxic cells, so they could be B lymphocytes, NK cells, or any other peripheral blood leukocyte.

Panel II, quadrant A (**choice E**) would contain cells which are strongly CD4+ and CD20–. These are helper T lymphocytes.

Panel II, quadrant B (**choice F**) would contain cells positive for CD4 and CD20. Because CD4 is a Th cell marker, and CD20 is a B-cell marker, such cells do not exist and thus this quadrant is empty.

Panel II, quadrant C (**choice G**) contains the cells that have only background levels of fluorescence with antibodies to CD4 and CD20. These would be nonhelper, non-B cells, so they could be cytotoxic T lymphocytes, NK cells, or any other peripheral blood leukocyte. Although this quadrant clearly contains some of the cytotoxic cells that this question asks about, there are other cells present, so this is not the best answer.

Panel II, quadrant D (**choice H**) contains the cells strongly positive for CD20 and negative for CD4. These are B lymphocytes.

PART II

Microbiology

General Microbiology 1

Learning Objectives

- ❑ Answer questions about bacterial structure, and bacterial growth and death
- ❑ Demonstrate knowledge about bacterial toxins

BACTERIAL STRUCTURE

Both Gram-positive and Gram-negative cell envelopes have the following properties:.

- **Cytoplasmic membranes**, which contain transpeptidases and carboxypeptidases that help construct the cell wall or peptidoglycan; they are also known as penicillin-binding proteins because they are targets for the β-lactam antibiotics
- **Peptidoglycan**, of which Gram-positives have a thick layer to protect it from osmotic damage, while Gram-negatives have a very thin layer; Gram stain reflects this difference

Gram-positive bacteria may utilize teichoic acid for attachment or lipoteichoic acid. Cell surface proteins are variable among the different genera but may include proteins such as the M protein found within the genus *Streptococcus*.

Gram-negative bacteria have an outer membrane covering the peptidoglycan. The outer membrane contains the endotoxin lipopolysaccharide (LPS).

- LPS is comprised of **lipid A (the toxic portion)**, and polysaccharide.
- LPS is highly immunogenic and binds specifically to receptors (LPS receptor, or **TLR-4**, see Immunology chapter 3) to activate macrophages.
- LPS can also non-specifically activate B cells without the help of T cells.
- LPS can be serotyped to classify bacteria.

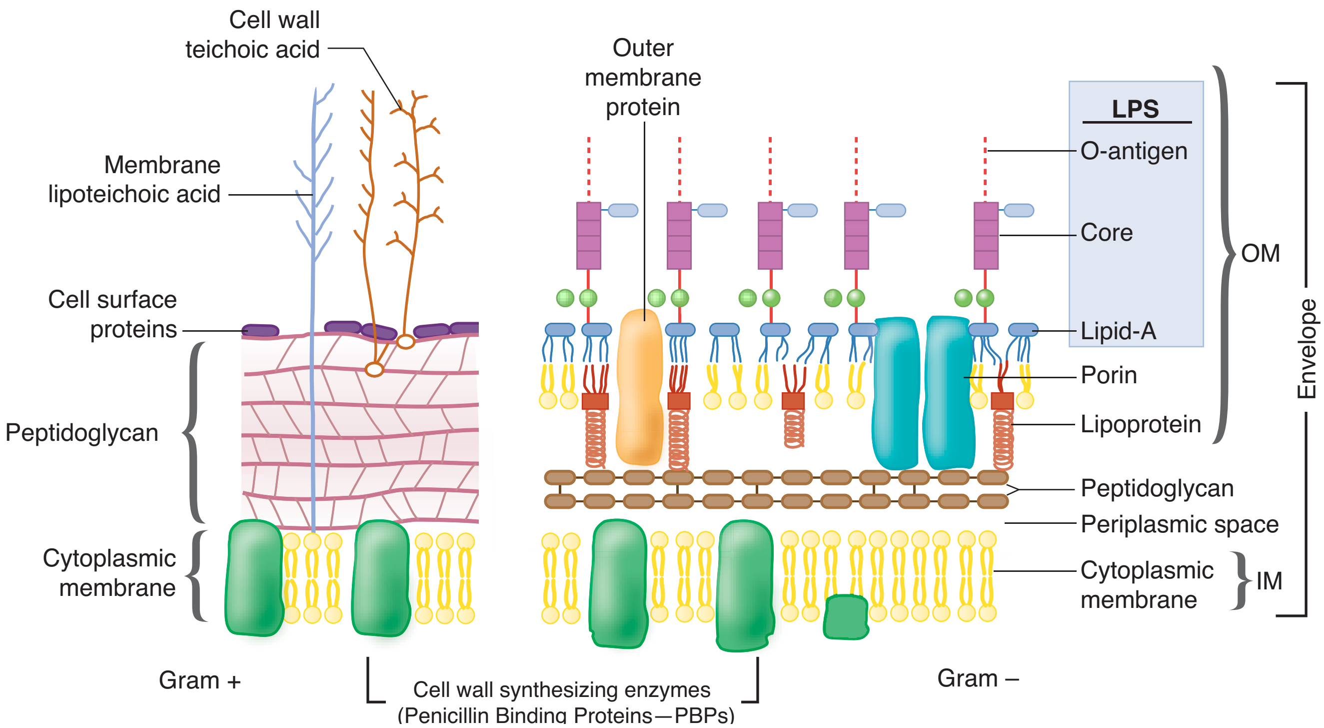


Figure II-1-1. Bacterial Cell Envelope

Peptidoglycan is comprised of polysaccharide chains N-acetylglucosamine (GlcNAc, NAG) and N-acetylmuramic acid (MurNAc, NAM) that are crosslinked by peptides. The peptide which crosslinks NAG and NAM is a tetrapeptide (begins as a pentapeptide but terminal D-alanine is released upon crosslinking to create the tetrapeptide) in which the first 2 amino acids may vary among different organisms.

The third amino acid in the peptide must include an amine group (e.g., L-lysine, diaminopimelic acid, diaminobutyric acid) which forms the cross link with the fourth peptide (D-alanine from another chain). The peptidoglycan is very thick in Gram positive cells and only 1 layer thick in gram negative cells.

Synthesis of the peptidoglycan is a several step process.

- The NAG and NAM precursors are synthesized inside of the cell and the pentapeptide is added.
- At the cytoplasmic membrane, the individual units are attached to a carrier called bactoprenol.
- The NAG is added and the unit (NAG-NAM-peptide) is translocated to the outside of the cell.
- The individual unit is then added to the peptidoglycan chain through transglycosylases which use a pyrophosphate link between the unit and the bactoprenol as energy to drive the reaction.

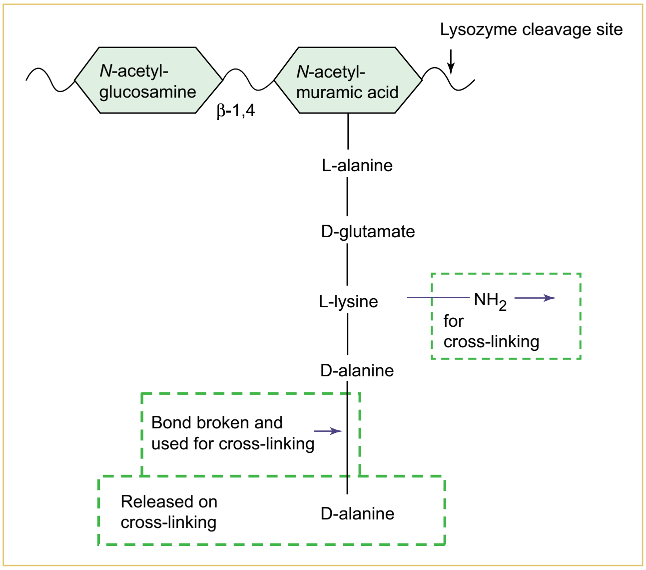


Figure II-1-2. Synthesis of Peptidoglycan

The cross-linking is catalyzed by transpeptidases and carboxypeptidases (remove the terminal D-alanines) that are also called penicillin-binding proteins (PBP's).

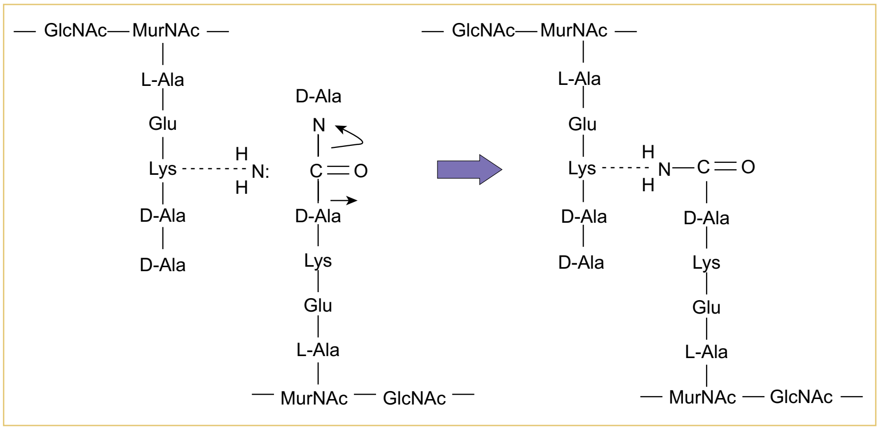


Figure II-1-3. Transpeptidation Reaction

Clinical Correlate

Peptidoglycan synthesis is a major target for antimicrobial chemotherapy for the following reasons:

- The process is utilized only in prokaryotes.
- Its inhibition has few side effects on eukaryotic cells (human cells).

As effective as antibiotics are, bacteria have become very clever at getting around them and becoming resistant to antibiotics.

Table II-1-1. Antibiotic MOA and Resistance Mechanisms

Antibiotic	Effect on PG synthesis	Resistance mechanisms
Bacitracin	Interferes with recycling of the pyrophosphobactoprenol back to phosphobactoprenol	
β-lactams	Target the PBPs	• Decreased binding of antibiotic to the PBP • Decreased concentration of antibiotic at target (Gram negative only) • β-lactamases
Vancomycin	Binds to D-ala-D-ala to block the transpeptidation reaction	Van-A and Van-B genes change the pentapeptide terminus so vancomycin will no longer bind

See Pharmacology Lecture Notes for more on the MOA of antibiotics.

The mechanism of action (MOA) of antibiotics is covered in the Pharmacology Lecture Notes but an overview can be seen below.

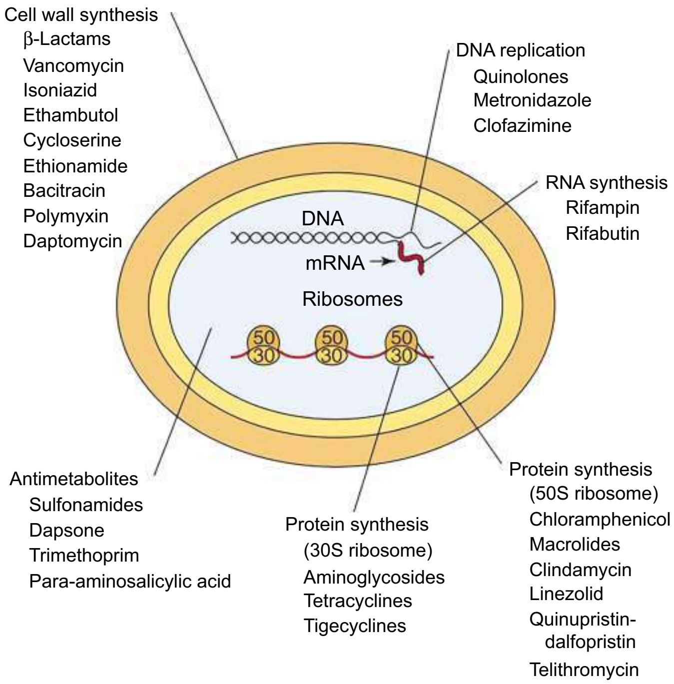


Figure II-1-4. Antibiotic MOA

ENDOSPORES

Organisms: *Bacillus* and *Clostridium*

Function: survival not reproductive (1 bacterium → 1 spore); resistance to chemicals, desiccation, radiation, freezing, and heat

Mechanism of resistance

- New enzymes (i.e., dipicolinic acid synthetase, heat-resistant catalase)
- Increases or decreases in other enzymes
- Dehydration: calcium dipicolinate in core
- Keratin spore coat

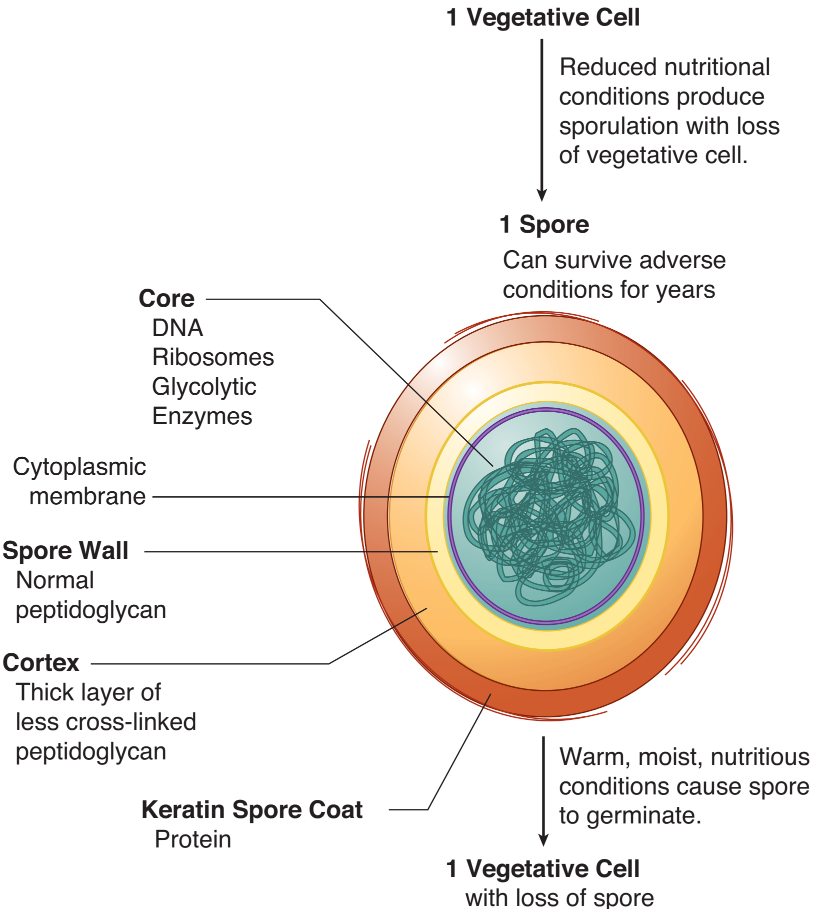


Figure II-1-5. Endospore

Note

Spores of fungi have a reproductive role.

BACTERIAL GROWTH AND DEATH

Note

Lag Phase

- Initial Phase (only 1 lag phase)
- Detoxifying medium
- Turning on enzymes to utilize medium
- For exam, number of cells at beginning equals number of cells at end of lag phase.

Log Phase

- Rapid exponential growth
- Generation time = time it takes one cell to divide into two. This is determined during log phase.

Stationary Phase

- Nutrients used up
- Toxic products like acids and alkali begin to accumulate.
- Number of new cells equals the number of dying cells.

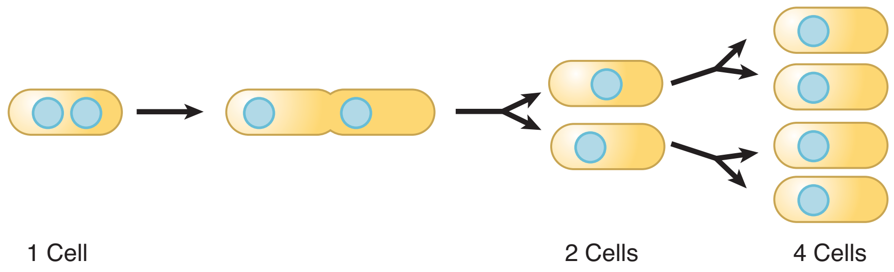


Figure II-1-6. Exponential Growth by Binary Fission

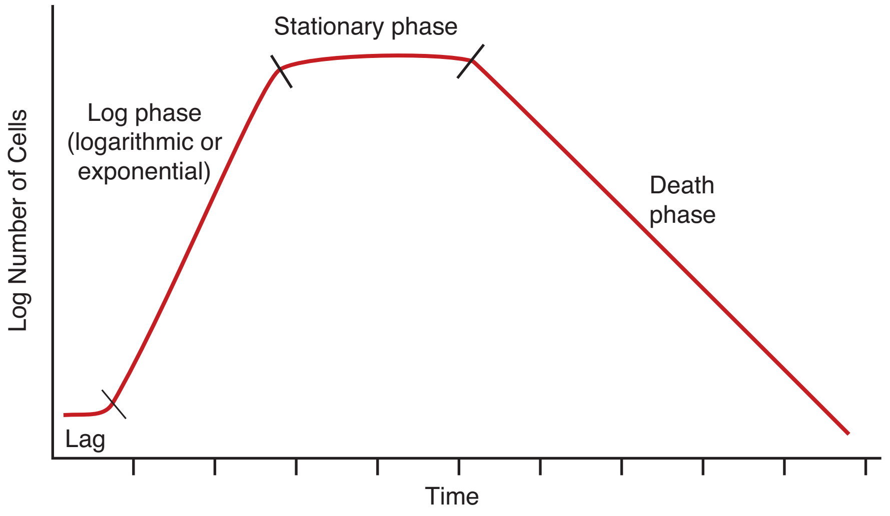


Figure II-1-7. Bacterial Growth Curve

Antibiotics are most effective at the logarithmic phase of the growth curve.

CULTURE OF MICROORGANISMS

Obligate intracellular pathogens (viruses, rickettsias, chlamydias, etc.) cannot be cultured in vitro on artificial media. Therefore, tissue cultures (cell cultures), eggs, animals are utilized for growth. Some pathogens cannot be cultured at all (e.g., syphilis).

Facultative intracellular or extracellular organisms can be grown on inert lab media such as broth or agar. Selective media selects for certain bacteria by inclusion of special nutrients and/or antibiotics. Differential media differentiates bacteria by colony morphology or color.

Table II-1-2. Special Media for Selected Organisms

Organism	Medium
Anaerobes	Thioglycolate
Corynebacterium	Löffler coagulated serum medium (S) Tellurite agar (D)
Enteric bacteria	Eosin methylene blue (D) MacConkey (D and S)
Enteric pathogens	Hektoen enteric agar (D) Xylose-lysine-deoxycholate agar
Vibrio cholerae (likes alkaline growth medium)	TCBS (Thiosulfate Citrate Bile Salts Sucrose agar) (S)
Legionella	Charcoal-yeast extract agar (CYE agar) (S)
Mycobacterium	Löwenstein-Jensen medium (S)
Neisseria from normally sterile sites, *Haemophilus*	Chocolate agar
Neisseria from sites with normal flora	Thayer-Martin selective medium* (S)

*Thayer-Martin media is a chocolate agar supplemented with vancomycin, nystatin and colistin to inhibit the normal flora, including nonpathogenic Neisseria.

Table II-1-3. Miscellaneous Growth Requirements

Cholesterol and purines and pyrimidines	*Mycoplasma*
Cysteine*	*Francisella, Brucella, Legionella, Pasteurella*
X (protoporphyrin) and V (NAD)	*Haemophilus* (*influenzae* and *aegyptius* require both)

*The 4 Sisters Ella and the Cysteine Chapel

Note

Mnemonic

The 4 sisters "Ella" worship in the "Cysteine" chapel:

- *Francisella*
- *Brucella*
- *Legionella*
- *Pasteurella*

PATHOGENICITY (INFECTIVITY AND TOXICITY) MAJOR MECHANISMS

Colonization

(Important unless organism is traumatically implanted.)

Adherence to cell surfaces involves

- **Pili/fimbriae:** primary mechanism in most gram-negative cells.
- **Teichoic acids:** primary mechanism of gram-positive cells.
- **Adhesins:** colonizing factor adhesins, pertussis toxin, and hemagglutinins.
- **IgA proteases:** make it easier for pathogens to colonize.

Partial adherence to inert materials, **biofilms**: *Staph. epidermidis, Streptococcus mutans, Pseudomonas aeruginosa*

Note

Mnemonic

***S**treptococcus pneumoniae*

***K**lebsiella pneumoniae*

***H**aemophilus influenzae*

***P**seudomonas aeruginosa*

***N**eisseria meningitidis*

***C**ryptococcus neoformans*

(**S**ome **K**illers **H**ave **P**retty **N**ice **C**apsules)

Avoiding Immediate Destruction by Host Defense System

- **Anti-phagocytic surface components** (inhibit phagocytic uptake):
  - **Capsules**/slime layers:

    ***Streptococcus pyogenes* M protein**

    ***Neisseria gonorrhoeae* pili**

    ***Staphylococcus aureus* A protein**
- **IgA proteases,** destruction of mucosal IgA: *Neisseria, Haemophilus, S. pneumoniae*

"Hunting and Gathering" Needed Nutrients

- **Siderophores** steal (chelate) and import iron.

Note

Intracellular organisms

- Elicit different immune responses
- Different pathology
- Different antibiotics
- Different culture techniques

Antigenic Variation

- Changing surface antigens to avoid immune destruction
- *N. gonorrhoeae*—pili and outer membrane proteins
- *Trypanosoma brucei rhodesiense* and *T. b. gambiense*—phase variation
- Enterobacteriaceae: capsular and flagellar antigens may or may not be expressed
- HIV, influenza—antigenic drift

Ability to Survive Intracellularly

- **Evading intracellular killing by professional phagocytic cells** allows intracellular growth:
  - *M. tuberculosis* survives by inhibiting phagosome-lysosome fusion.
  - *Listeria* quickly escapes the phagosome into the cytoplasm **before** phagosome-lysosome fusion.
- **Invasins**: surface proteins that allow an organism to bind to and invade normally non-phagocytic human cells, escaping the immune system. Best studied invasin is on *Yersinia pseudotuberculosis* (an organism causing diarrhea).
- Damage from viruses is largely from intracellular replication, which either kills cells, transforms them or, in the case of latent viruses, may do no noticeable damage.

Type III Secretion Systems

- Tunnel from the bacteria to the host cell (macrophage) that delivers bacterial toxins directly to the host cell
- Have been demonstrated in many pathogens: *E. coli, Salmonella* species, *Yersinia* species, *P. aeruginosa*, and *Chlamydia*

Inflammation or Immune-Mediated Damage

Examples

- **Cross-reaction of bacteria-induced antibodies with tissue antigens** causes disease. Rheumatic fever is one example.
- **Delayed hypersensitivity and the granulomatous response** stimulated by the presence of intracellular bacteria is responsible for neurological damage in leprosy, cavitation in tuberculosis, and fallopian tube blockage resulting in infertility from *Chlamydia* PID (pelvic inflammatory disease).
- **Immune complexes** damage the kidney in post streptococcal acute glomerulonephritis.
- **Peptidoglycan-teichoic acid** (large fragments) of gram-positive cells:
  - Serves as a structural toxin released when cells die.
  - Chemotactic for neutrophils.

Physical Damage

- Swelling from infection in a fixed space damages tissues; examples: meningitis and cysticercosis.
- Large physical size of organism may cause problems; example: *Ascaris lumbricoides* blocking bile duct.

TOXINS

Toxins may aid in invasiveness, damage cells, inhibit cellular processes, or trigger immune response and damage.

Structural Toxins

- **Endotoxin** (lipopolysaccharide = LPS)
  - LPS is part of **gram-negative outer membrane**
  - **Toxic portion is lipid A**: generally not released (and toxic) until death of cell (exception: *N. meningitidis,* which over-produces outer membrane fragments)
  - **LPS is heat stable** and not strongly immunogenic, so **cannot be converted to a toxoid**
  - **LPS is primary virulence factor in Gram negative septic shock**
  - Mechanism: **LPS activates macrophages**, leading to release of TNF-alpha, IL-1, and IL-6. IL-1 is a major mediator of fever. Macrophage activation and products lead to tissue damage. Damage to the endothelium from **bradykinin-induced vasodilation leads to shock**. **Coagulation** (DIC) is mediated through the **activation of Hageman factor**.
- **Peptidoglycan, Teichoic Acids**

Exotoxins

- Are **protein toxins,** generally **quite toxic** and **secreted by** bacterial **cells** (some gram +, some gram –)
- **Can be modified** by chemicals or heat to produce a **toxoid** that still is **immunogenic, but no longer toxic** so can be used as a vaccine
- **A-B** (or "two") **component** protein toxins
  - **B** component **binds** to specific cell receptors to facilitate the internalization of A.
  - **A** component is the **active (toxic) component** (often an enzyme such as an ADP ribosyl transferase).
  - Exotoxins may be subclassed as enterotoxins, neurotoxins, or cytotoxins.
- **Cytolysins:** lyse cells from outside by damaging membrane.
  - ***C. perfringens*** **alpha toxin** is a **lecithinase.**
  - ***Staphylococcus aureus*** **alpha toxin** inserts itself to form **pores** in the membrane.

Table II-1-4. Major Exotoxins

	Organism (Gram)	Toxin	Mode of Action	Role in Disease
Inhibitors of Protein Synthesis	*Corynebacterium diphtheriae* (+)	Diphtheria toxin	ADP ribosyl transferase; inactivates eEF-2; 1´ targets: heart/ nerves/epithelium	Inhibits eukaryotic cell protein synthesis
	Pseudomonas aeruginosa (–)	Exotoxin A	ADP ribosyl transferase; inactivates eEF-2; 1´ target: liver	Inhibits eukaryotic cell protein synthesis
	Shigella dysenteriae (–)	Shiga toxin	Interferes with 60S ribosomal subunit	Inhibits protein synthesis in eukaryotic cells. Enterotoxic, cytotoxic, and neurotoxic
	Enterohemorrhagic *E. coli* (EHEC) (–)	Verotoxin (a shiga-like toxin)	Interferes with 60S ribosomal subunit	Inhibits protein synthesis in eukaryotic cells
Neurotoxins	*Clostridium tetani* (+)	Tetanus toxin	Blocks release of the inhibitory transmitters glycine and GABA	Inhibits neurotransmission in inhibitory synapses
	Clostridium botulinum (+)	Botulinum toxin	Blocks release of acetylcholine	Inhibits cholinergic synapses
Super-antigens	*Staphylococcus aureus* (+)	TSST-1	Superantigen	Fever, increased susceptibility to LPS, rash, shock, capillary leakage
	Streptococcus pyogenes (+)	Exotoxin A, a.k.a.: erythrogenic or pyrogenic toxin	Similar to TSST-1	Fever, increased susceptibility to LPS, rash, shock, capillary leakage, cardiotoxicity
cAMP Inducers	Enterotoxigenic *Escherichia coli* (–)	Heat labile toxin (LT)	LT stimulates an adenylate cyclase by ADP ribosylation of GTP binding protein	Both LT and ST promote secretion of fluid and electrolytes from intestinal epithelium
	Vibrio cholerae (–)	Cholera toxin	Similar to *E. coli* LT	Profuse, watery diarrhea
	Bacillus anthracis (+)	Anthrax toxin (3 proteins make 2 toxins)	EF = edema factor = adenylate cyclase LF = lethal factor PA = protective antigen (B component for both)	Decreases phagocytosis; causes edema, kills cells
	Bordetella pertussis (–)	Pertussis toxin	ADP ribosylates G_i, the negative regulator of adenylate cyclase → increased cAMP	Histamine-sensitizing Lymphocytosis promoting Islet activating
Cytolysins	*Clostridium perfringens* (+)	Alpha toxin	Lecithinase	Damages cell membranes; myonecrosis
	Staphylococcus aureus (+)	Alpha toxin	Toxin intercalates forming pores	Cell membrane becomes leaky

Medically Relevant Bacteria 2

Learning Objectives

- ❑ Answer questions about normal flora and their function
- ❑ Demonstrate understanding of microorganism cultures, strains, and gram stain techniques
- ❑ Answer questions about distinguishing features, transmission, pathogenesis, diagnosis, treatment, and prevention of gram-positive cocci, gram-positive rods, gram-negative cocci, gram-negative bacilli, spirochetes, and unusual bacteria
- ❑ Differentiate organisms in the *Chlamydophila*, *Rickettsia*, and *Ehrlichia* genuses
- ❑ Differentiate organisms in the *Mycoplasmataceae* and *Chlamydiaceae* families

NORMAL FLORA

Normal floras are found on body surfaces contiguous with the outside environment. They are semi-permanent, varying with major life changes.

- Can cause infection
  - If misplaced, e.g., fecal flora to urinary tract or abdominal cavity, or skin flora to catheter
  - If person becomes compromised, normal flora may overgrow (oral thrush)
- Contribute to health
  - Protective host defense by maintaining conditions such as pH so other organisms may not grow
  - Serve nutritional function by synthesizing: K and B vitamins
  - Competition for space

Note

Nomenclature

Latin bacterial **family** names have **"-aceae,"** e.g., Enterobacteriaceae.

Genus and species names are **italicized** and **abbreviated**, e.g., *Enterobacter aerogenes* = *E. aerogenes*.

Note

Definitions

Carrier: person colonized by a potential pathogen without overt disease.

Bacteremia: bacteria in bloodstream without overt clinical signs.

Septicemia: bacteria in bloodstream (multiplying) with clinical symptoms.

Table II-2-1. Important Normal Flora

Site	Common or Medically Relevant Organisms	Less Common but Notable Organisms
Blood, internal organs	None, generally sterile	
Cutaneous surfaces including urethra and outer ear	*Staphylococcus epidermidis*	*Staphylococcus aureus*, Corynebacteria (diphtheroids), streptococci, anaerobes, e.g., peptostreptococci, yeasts (*Candida* spp.)
Nose	*Staphylococcus aureus*	*S. epidermidis*, diphtheroids, assorted streptococci
Oropharynx	**Viridans streptococci** including *Strep. mutans*[1]	Assorted streptococci, **nonpathogenic *Neisseria*, nontypeable[2] *Haemophilus influenzae, Candida albicans***
Gingival crevices	Anaerobes: *Bacteroides, Prevotella, Fusobacterium, Streptococcus, Actinomyces*	
Stomach	None	
Colon (microaerophilic/anaerobic)	Babies; breast-fed only: *Bifidobacterium*	*Lactobacillus*, streptococci
	Adult: ***Bacteroides***/*Prevotella* (Predominant organism) *Escherichia* *Bifidobacterium*	*Eubacterium, Fusobacterium, Lactobacillus*, assorted Gram-negative anaerobic rods, *Enterococcus faecalis* and other streptococci
Vagina	***Lactobacillus***[3]	Assorted streptococci, gram-negative rods, diphtheroids, yeasts, *Veillonella*

[1]***S. mutans*** secretes a biofilm that glues it and other oral flora to teeth, producing **dental plaque**.

[2]Nontypeable for *Haemophilus* means no capsule.

[3]Group B streptococci colonize vagina of 15–20% of women and may infect the infant during labor or delivery, causing septicemia and/or meningitis (as may *E. coli* from fecal flora).

Table II-2-2. Oxygen Requirements and Toxicity

Classification	Characteristics	Important Genera
Obligate aerobes	Require oxygen Have no fermentative pathways Generally produce superoxide dismutase	*Mycobacterium* *Pseudomonas* *(Bacillus)*
Microaerophilic	Require low but not full oxygen tension	*Campylobacter* *Helicobacter*
Facultative anaerobes	Will respire aerobically until oxygen is depleted and then ferment	Most bacteria, e.g., *Enterobacteriaceae*
Obligate anaerobes	• Lack superoxide dismutase • Generally lack catalase • Are fermenters • Cannot use O_2 as terminal electron acceptor	*Actinomyces** *Bacteroides* *Clostridium*

*ABCs of anaerobiosis = *Actinomyces*, *Bacteroides*, and *Clostridium*

STAINS

Table II-2-3. Gram Stain

Reagent	Gram-Positive	Gram-Negative
Crystal violet (a very intense purple, small dye molecule)	Purple/blue	Purple/blue
Gram's iodine	Purple/blue (a large dye complex)	Purple/blue (a large dye complex)
Acetone or alcohol	Purple/blue	Colorless
Safranin (a pale dye)	Purple/blue	Red/pink

All cocci are gram-positive except *Neisseria*, *Moraxella* and *Veillonella*.
All spore formers are gram-positive.
Background in stain modified for tissues will be pale red.

Table II-2-4. Ziehl-Neelsen Acid Fast Stain (or Kinyoun)

Reagent	Acid Fast	Non-Acid Fast*
Carbol fuchsin with heat**	Red (hot pink)	Red (hot pink)
Acid alcohol	Red	Colorless
Methylene blue***	Red	Blue

* *Mycobacterium* is acid fast. *Nocardia* is partially acid fast. All other bacteria are non-acid fast. Two protozoan parasites (*Cryptosporidium* and *Isospora*) have acid fast oocysts.

** Without the heat, the dye would not go in the mycobacterial cells.

*** Sputa and human cells will be blue.

GRAM-STAINING REACTIONS

Table II-2-5. Gram-Negative Bacteria

Cocci		
	*Neisseria*** *Moraxella*	
Rods		
	*Pseudomonas*** *Burkholderia* *Acinetobacter* *Eikenella* *Kingella* *Haemophilus*** *Bartonella*	*Legionella** *Brucella* *Bordetella** *Francisella** *Capnocytophaga* *Pasteurella**
	Curved or helical	
	*Campylobacter*** *Helicobacter*** *Vibrio***	
	Enterobacteriaceae	
	*Escherichia*** *Shigella*** *Salmonella*** *Klebsiella** *Proteus**	*Yersinia*** *Aeromonas* *Serratia* *Citrobacter* *Enterobacter*
	Anaerobic Rods	
	Bacteroides *Prevotella*	*Porphyromonas* *Fusobacterium*
	Gram-variable Rods	
	Gardnerella	*Mobiluncus*
	Spirochetes	
	*Treponema*** *Borrelia*** *Leptospira*	

Table II-2-6. Gram-Positive Bacteria

Cocci	
	*Staphylococcus***
	*Streptococcus***
	Enterococcus
Rods	
Aerobic or facultative anaerobic	
	*Bacillus***
	*Listeria**
	*Corynebacterium**
	Nocardia
	*Mycobacterium***
Anaerobic	
	*Clostridium***
	Actinomyces
	Propionibacterium
	Lactobacillus

Note: Spore formers are *Bacillus* and *Clostridium*.

Note

*Typically considered high yield

** Extremely high yield organisms

Table II-2-7. Non-Gram–staining Bacteria*

Mycoplasmataceae	
	*Mycoplasma*** *Ureaplasma*
Rickettsiaceae	
	*Rickettsia** *Ehrlichia* *Coxiella*
Chlamydiaceae	
	*Chlamydia*** *Chlamydophila*

*Note:

Poorly visible on traditional Gram stain: ***Mycobacterium*** does not stain well with the Gram stain due to its waxy cell wall. It is considered gram-positive.

Most **spirochetes, chlamydiae**, and **rickettsias** are so thin that the color of the Gram stain cannot be seen. All have gram-negative cell walls.

Legionella (gram-negative) also does not stain well with the traditional Gram stain unless counterstain time is increased.

GRAM-POSITIVE COCCI

- *Staphylococcus*
- *Streptococcus*

Table II-2-8. Major Species of *Staphylococcus* and *Streptococcus* and Identifying Features*

	Catalase	Coagulase	Hemolysis†	Distinguishing Features	Disease Presentations
***Staphylococcus* Species**					
S. aureus	+	+	β	Ferments mannitol Salt tolerant	Infective endocarditis (acute) Abscesses Toxic shock syndrome Gastroenteritis Suppurative lesions, pyoderma, impetigo Osteomyelitis
S. epidermidis	+	–	γ	Novobiocin^S Biofilm producer	Endocarditis in IV drug users Catheter and prosthetic device infections
S. saprophyticus	+	–	γ	Novobiocin^R	UTIs in newly sexually active females
***Streptococcus* Species (Grouped by analysis of C carbohydrate)**					
S. pyogenes (Group A)	–	–	β	Bacitracin^S PYR†	Pharyngitis Scarlet fever Pyoderma/impetigo Suppurative lesions Rheumatic fever Acute glomerulonephritis
S. agalactiae (Group B)	–	–	β	Bacitracin^R CAMP+	Neonatal septicemia and meningitis
S. pneumoniae (not groupable)	–	–	α	Optochin^S Bile-soluble	Pneumonia (community acquired) Adult meningitis Otitis media and sinusitis in children
Viridans group (not groupable)	–	–	α/γ	Optochin^R Bile-soluble	Infective endocarditis Dental caries
Enterococcus sp. (Group D)	–	–	α, β, or γ	PYR† Bile-soluble Esculin agar	Infective endocarditis Urinary and biliary infections

† β hemolysis = clear; α hemolysis = partial; γ hemolysis = no hemolysis

Definition of abbreviations: PYR, pyrrolidonyl arylamidase; ^{S}, sensitive; ^{R}, resistant

*Many of the diseases caused by *Staphylococcus* and *Streptococcus* are similar (i.e., skin infections, endocarditis). Therefore, laboratory tests are extremely important in differentiating between these organisms.

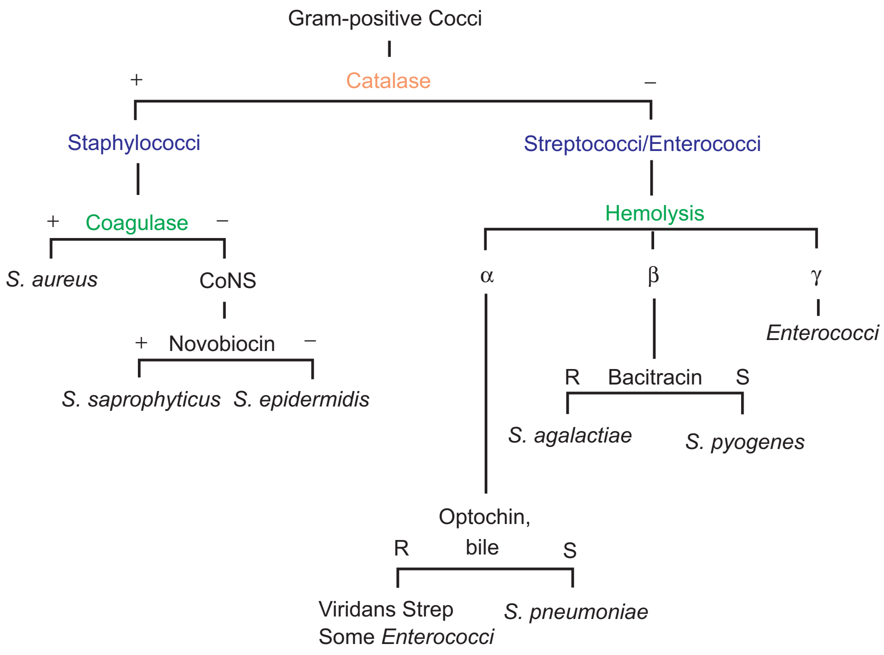


Figure II-2-1. Differentiation of Gram-Positive Cocci

GENUS: *STAPHYLOCOCCUS*

Genus Features

- Gram-positive cocci in clusters
- **Catalase positive (streptococci are catalase negative)**

Species of Medical Importance

- *S. aureus*
- *S. epidermidis*
- *S. saprophyticus*

Staphylococcus aureus

Distinguishing Features

- Small, yellow Staphylococcus aureuscolonies on blood agar
- **β-hemolytic**
- **Coagulase positive** (all other *Staphylococcus* species are negative)
- **Ferments mannitol** on mannitol salt agar

Reservoir

- Normal flora
  - Nasal mucosa (25% of population are carriers)
  - Skin

Key Vignette Clues

S. epidermidis

- Coagulase (–); gram (+) cocci
- Novobiocin sensitive
- Infections of catheters/shunts

S. saprophyticus

- Coagulase (–), gram (+) cocci
- Novobiocin resistant

Key Vignette Clues

Staphylococcus aureus

- Coagulase (+), gram (+) cocci in clusters
- Gastroenteritis: 2–6 h onset, salty foods, custards
- Endocarditis: acute
- Toxic shock syndrome: desquamating rash, fever, hypotension
- Impetigo: bullous
- Pneumonia: nosocomial, typical, acute
- Osteomyelitis: #1 cause unless HbS mentioned

Transmission

- Hands
- Sneezing
- Surgical wounds
- Contaminated food
  - Custard pastries
  - Potato salad
  - Canned meats

Predisposing Factors for Infection

- Surgery/wounds
- Foreign body (tampons, surgical packing, sutures)
- Severe neutropenia (<500/μL)
- Intravenous drug abuse
- Chronic granulomatous disease
- Cystic fibrosis

Pathogenesis

- Protein A binds Fc component of IgG, inhibits phagocytosis
- **Enterotoxins:** fast acting, heat stable
- **Toxic shock syndrome toxin-1 (TSST-1)**: superantigen (see Chapter 6 of Immunology for further explanation of a superantigen)
- Coagulase: converts fibrinogen to fibrin clot
- Cytolytic toxin (α toxin): pore-forming toxin, Panton-Valentine leukocidin (PVL), forms pores in infected cells and is acquired by bacteriophage; associated with increased virulence, MRSA strains
- Exfoliatins: skin-exfoliating toxins (involved in scalded skin syndrome [SSS]) and bullous impetigo

Diseases

Table II-2-9. *Staphylococcus aureus*

Diseases	Clinical Symptoms	Pathogenicity Factors
Gastroenteritis (food poisoning)—toxin ingested preformed in food	**2–6 hours** after ingesting toxin: nausea, abdominal pain, vomiting, followed by diarrhea	Enterotoxins A–E preformed in food
Infective endocarditis (acute) (**most common cause**)	Fever, malaise, leukocytosis, heart murmur (may be absent initially)	Fibrin-platelet mesh, cytolytic toxins
Abscesses and mastitis	Subcutaneous tenderness, redness and swelling; hot	Coagulase, cytolysins
Toxic shock syndrome	Fever, hypotension, **scarlatiniform rash that desquamates (particularly on palms and soles)**, multiorgan failure	TSST-1
Impetigo	Erythematous papules to **bullae**	Coagulase, exfoliatins
Scalded skin syndrome	Diffuse epidermal peeling	Coagulase, exfoliatins
Pneumonia	Productive pneumonia with rapid onset, high rate of necrosis, and high fatality; nosocomial, ventilator, postinfluenza, IV drug abuse, cystic fibrosis, chronic granulomatous disease, etc. Salmon-colored sputum	Coagulase, cytolysins
Surgical infections	Fever with cellulitis and/or abscesses	Coagulase, exfoliatins, ± TSSTs
Osteomyelitis (most common cause)	Bone pain, fever, ± tissue swelling, redness; lytic bone lesions on imaging	Cytolysins, coagulase
Septic arthritis	Monoarticular joint pain; inflam-mation	Cytolysins, coagulase

Treatment

- Gastroenteritis is self-limiting.
- Nafcillin/oxacillin are drugs of choice because of widespread penicillinase-producing stains.
- Mupirocin for topical treatment.
- For methicillin-resistant *Staphylococcus aureus* (MRSA): vancomycin
- For vancomycin-resistant *Staphylococcus aureus* (VRSA) or vancomycin-intermediate *S. aureus* (VISA): quinupristin/dalfopristin

GENUS: *STREPTOCOCCUS*

Genus Features

- Gram-positive cocci in chains
- **Catalase negative**
- Serogrouped using known antibodies to the cell wall carbohydrates (Lancefield groups A–O): *S. pneumoniae* serotyped via capsule; *S. pyogenes* serotyped via M protein

Species of Medical Importance

- *S. pyogenes*
- *S. agalactiae* (group B streptococci; GBS)
- *S. pneumoniae*
- Viridans streptococci: *S. mutans; S. sanguinis; S. gallolyticus (bovis)*

Streptococcus pyogenes (Group Enterococcus Streptococcus; GAS)

Distinguishing Features

- **β hemolytic**
- **Bacitracin sensitive**
- **Pyrrolidonyl arylamidase (PYR) positive**

Reservoir: human throat; skin

Transmission: direct contact; respiratory droplets

Pathogenesis

- **Hyaluronic acid: is non-immunogenic**
- **M-protein: antiphagocytic**, associated with **acute glomerulonephritis**, rheumatic fever
- Streptolysin O: immunogenic, hemolysin/cytolysin
- Streptolysin S: not immunogenic, hemolysin/cytolysin

Key Vignette Clues

Group A *Streptococcus*

- Catalase (–), β hemolytic, bacitracin sensitive, gram (+) cocci
- Pharyngitis: abrupt onset, tonsillar abscesses
- Scarlet fever: blanching, sandpaper rash, strawberry tongue
- Impetigo: honey-crusted lesions

Key Vignette Clues

- Rheumatic fever: after streptococcal pharyngitis, ↑ ASO titer
- AGN: after streptococcal skin or throat infection, hypertension, edema, smoky urine

Spreading Factors

- Streptokinase: breaks down fibrin clot
- Streptococcal DNAse: liquefies pus, extension of lesion
- Hyaluronidase: hydrolyzes the ground substances of the connective tissues
- Exotoxins A–C (pyrogenic or erythrogenic exotoxins)
  - Phage-coded (i.e., the cells are lysogenized by a phage)
  - Cause **fever and rash** of scarlet fever: **superantigens**

Diseases

Table II-2-10. Acute Suppurative Group A Streptococcal Infections*

Diseases	Symptoms
Pharyngitis	Abrupt onset of sore throat, fever, malaise, and headache; tonsillar abscesses and tender anterior cervical lymph nodes
Scarlet fever	Above followed by a blanching **"sandpaper" rash** (palms and soles are usually spared), circumoral pallor, **strawberry tongue**, and nausea/vomiting
Pyoderma/impetigo	Pyogenic skin infection (honey-crusted lesions)

*Also, cellulitis/necrotizing fasciitis, puerperal fever, lymphangitis, erysipelas

Table II-2-11. Nonsuppurative Sequelae to Group A Streptococcal Infections

Disease	Sequelae of	Mechanisms/Symptoms
Rheumatic fever	Pharyngitis with group A strep	Antibodies to heart tissue/2 weeks post pharyngitis, fever, joint inflammation, carditis, erythema marginatum (chorea later)type II hypersensitivity
Acute glomerulonephritis (AGN)	Pharyngitis or skin infection	Immune complexes bound to glomeruli/pulmonary edema and hypertension, "smoky" urine (type III hypersensitivity)

Laboratory Diagnosis

- Rapid strep test (ELISA-based) misses approximately 25% of infections. Culture all negatives.
- Antibodies to streptolysin O (ASO) titer of >200 is significant for rheumatic fever.
- Anti-DNAse B and antihyaluronidase titers for AGN

Treatment: beta lactam drugs, macrolides in the case of penicillin allergy

Prevention: possible prophylactic antibiotics for at least 5 years post-acute rheumatic fever; beta lactams and macrolides

Streptococcus agalactiae (Group B Streptococci; GBS)

Key Vignette Clues

S. agalactiae

- Gram (+), catalase (–), β hemolytic, bacitracin resistant, CAMP test (+)
- Neonatal meningitis and septicemia: #1 cause, especially in prolonged labors

Distinguishing Features

- β **hemolytic**
- **Bacitracin resistant**
- **Hydrolyze hippurate**
- **CAMP test positive** (CAMP factor is a polypeptide which "complements" the sphingomyelinase of *S. aureus* to create an enhanced hemolytic pattern in shape of an arrowhead)

Reservoir: human vagina (15–20% of women); GI tract

Transmission: newborn infected during birth (increased risk with **prolonged labor after rupture of membranes**)

Pathogenesis: capsule; β hemolysin and CAMP factor

Diseases: neonatal septicemia and meningitis; most common causal agent

Treatment: ampicillin with an aminoglycoside or a cephalosporin

Prevention

- Prophylaxis during delivery in women with positive vaginal/rectal culture of GBS, history of recent infection with GBS, or prolonged labors after membrane rupture
- Ampicillin or penicillin drugs of choice
- Clindamycin or erythromycin for penicillin allergies

Streptococcus pneumoniae

Distinguishing Features

- α **hemolytic**
- **Optochin sensitive**
- **Lancet-shaped diplococci**
- **Lysed by bile** (bile soluble)

Reservoir: human upper respiratory tract

Transmission: respiratory droplets (not considered highly communicable; often colonize the nasopharynx without causing disease)

Predisposing Factors

- Antecedent influenza or measles infection
- Chronic obstructive pulmonary disease (COPD)
- Congestive heart failure (CHF)
- Alcoholism
- Asplenia predisposes to septicemia

Pathogenesis

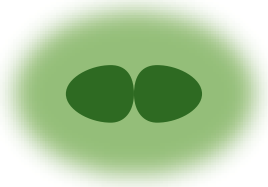
- **Polysaccharide capsule** is the major virulence factor
- IgA protease
- Teichoic acid
- Pneumolysin O: hemolysin/cytolysin: damages respiratory epithelium; inhibits leukocyte respiratory burst and inhibits classical complement fixation

Diseases

- Typical pneumonia: **most common cause** (especially in decade 6 of life); shaking chills, high fever, lobar consolidation, **blood-tinged, "rusty" sputum**
- Adult meningitis: **most common cause**; peptidoglycan and teichoic acids are highly inflammatory in CNS; CSF reveals high WBCs (neutrophils) and low glucose, high protein
- Otitis media and sinusitis in children **most common cause**

Laboratory Diagnosis

- Gram stain and culture of CSF or sputum
- Quellung reaction: positive (swelling of the capsule with the addition of type-specific antiserum, no longer used but still tested!)
- Latex particle agglutination: test for capsular antigen in CSF
- Urinary antigen test



Streptococcus pneumoniae

Key Vignette Clues

Streptococcus pneumoniae

- Gram (+), catalase (–), α hemolytic, soluble in bile, optochin sensitive
- Pneumonia—typical, most common cause, rusty sputum
- Meningitis—many PMNs, ↓ glucose, ↑ protein in CSF, most common adult cause
- Otitis media and sinusitis—most common cause

Note

Typical Pneumonia

Bacterial pneumonia such as *Streptococcus pneumoniae* elicits neutrophils; arachidonic acid metabolites (acute inflammatory mediators) cause pain and fever. *Pneumococcus* produces a lobar pneumonia with a productive cough, grows on blood agar, and usually responds well to penicillin treatment.

Treatment: beta lactams for bacterial pneumonia; ceftriaxone or cefotaxime for adult meningitis (add vancomycin if penicillin-resistant *S. pneumoniae* has been reported in community); amoxicillin for otitis media and sinusitis in children (erythromycin in cases of allergy)

Prevention

- Antibody to capsule (>80 capsular serotypes) provides type-specific immunity
- Vaccine
  - Pediatric (PCV, pneumococcal conjugate vaccine): 13 of most common serotypes; conjugated to diphtheria toxoid; prevents invasive disease
  - Adult (PPV, pneumococcal polysaccharide vaccine): 23 of most common capsular serotypes; recommended for all adults age ≥65 plus at-risk individuals

Key Vignette Clues

Viridans Streptococci

- Gram (+), catalase (–), α hemolytic, optochin resistant, bile insoluble
- Plaque and dental caries
- Subacute bacterial endocarditis—preexisting damage to heart valves; follows dental work

Viridans Streptococci (*S. sanguis*, *S. mutans*)

Distinguishing Features

- α **hemolytic**
- **Optochin resistant**
- PYR-negative
- Bile insoluble

Reservoir: normal flora of human oropharynx (*S. mutans*, *S. sanguinis*), human colon (*S. gallolyticus*)

Transmission: endogenous

Pathogenesis: **dextran (biofilm)-mediated adherence** onto tooth enamel or damaged heart valve and to each other (vegetation); growth in vegetation protects organism from immune system

Diseases

- Dental caries: *S. mutans* dextran-mediated adherence glues oral flora onto teeth, forming plaque and causing dental caries
- Infective endocarditis (subacute): m
  - Malaise, fatigue, anorexia, night sweats, weight loss, splinter hemorrhages
  - Predisposing conditions: damaged (or prosthetic) heart valve *and* dental work without prophylactic antibiotics or extremely poor oral hygiene
  - *S. gallolyticus* associated with colon cancer

Treatment: penicillin G with aminoglycosides for endocarditis

Prevention: prophylactic antibiotics prior to dental work for individuals with damaged heart valve

GENUS: *ENTEROCOCCUS*

Genus Features

- **Catalase negative**
- **PYR+**

Species of Medical Importance

- *Enterococcus faecalis*
- *Enterococcus faecium*

Enterococcus faecalis/faecium

Distinguishing Features

- **Group D gram-positive cocci in chains**
- **PYR test positive**
- Catalase-negative, varied hemolysis
- **Hydrolyze esculin in 40% bile and 6.5% NaCl** (bile esculin agar turns black)

Reservoir: human colon, urethra ± and female genital tract

Transmission: endogenous

Pathogenesis

- Bile/salt tolerance allows survival in bowel and gall bladder.
- During medical procedures on GI or GU tract: ***E. faecalis*** → **bloodstream** → **previously damaged heart valves** → **endocarditis**

Diseases: urinary and biliary tract infection; infective (subacute) endocarditis in persons (often elderly) with damaged heart valves

Diagnosis

- Culture on blood agar
- Antibiotic sensitivities

Treatment: all strains carry some drug resistance

- Some **vancomycin-resistant strains of *Enterococcus faecium*** or ***E. faecalis*** have no reliably effective treatment; or low-level resistance use ampicillin, gentamicin, or streptomycin
- VanA strains have UDP-N-acetylmuramyl pentapeptide with terminal D-alanyl-D-alanine replaced with D-alanyl-D-lactate, which functions in cell wall synthesis but does not bind to vancomycin

Prevention: prophylactic use of penicillin and gentamicin for patients with damaged heart valves prior to intestinal or urinary tract manipulation

Key Vignette Clues

Enterococcus faecalis/faecium

- Gram (+), catalase (–), variable hemolysis, hydrolyzes esculin
- Urinary/biliary tract infections—elderly males
- Subacute bacterial endocarditis—elderly males, follows GI/GU surgery, preexisting heart valve damage

GRAM-POSITIVE RODS

Table II-2-12. Gram-Positive Rods

Genus	Spore	Aerobic Growth	Exotoxin	Facultative Intracellular	Acid Fast	Branching Rods
Bacillus	+	+	+	–	–	–
Clostridium	+	–	+	–	–	–
Listeria	–	+	–	+	–	–
Corynebacterium	–	+	+	–	–	–
Actinomyces	–	–	–	–	–	+
Nocardia	–	+	–	–	+†	+
Mycobacterium	–	+	–	+	+	–

†*Nocardia* is considered partially acid fast.

GENUS: *BACILLUS*

Bacillus anthracis

Key Vignette Clues

Bacillus anthracis

- Gram (+), spore forming, aerobic rods
- Contact with animal hides or bioterrorism; eschar or life-threatening pneumonia

Distinguishing Features

- **Large,** boxcar-like, gram-positive, **spore-forming rods**
- **Capsule is polypeptide (poly-d-glutamate)**
- **Potential bioterrorism agent**

Reservoir: animals, skins, soils

Transmission: contact with infected animals or inhalation of spores (bioterrorism)

Pathogenesis

- Capsule polypeptide, antiphagocytic, immunogenic
- **Anthrax toxin** includes 3 protein components:
  - **Protective antigen** (B component) mediates entry of LF or EF into eukaryotic cells
  - **Lethal factor** kills cells
  - **Edema factor** is an adenylate cyclase (calmodulin-activated like pertussis adenylate cyclase)

Diseases

- Cutaneous anthrax: papule → papule with vesicles (malignant pustules) → central necrosis (eschar) with erythematous border often with painful regional lymphadenopathy; fever in 50%
- Pulmonary (woolsorter's disease): life-threatening **pneumonia**; cough, fever, malaise, and ultimately facial edema, dyspnea, diaphoresis, cyanosis, and shock with **mediastinal hemorrhagic lymphadenitis**
- GI anthrax (rare): edema and blockage of G tract can occur, vomiting and bloody diarrhea, high mortality

Diagnosis

- Mediastinal widening on chest x-ray
- Gram stain and culture of blood, respiratory secretions or lesions
- Serology
- PCR

Treatment: ciprofloxacin or doxycycline. (Genes encoding resistance to penicillin and doxycycline have been transferred to *B. anthracis*.)

Prevention: toxoid vaccine (AVA, acellular vaccine adsorbed) is given to those in high risk occupations such as military; raxibacumab for prophylaxis

Bacillus cereus

Distinguishing Feature: spores

Reservoir: found in nature

Transmission

- Foodborne, intoxication
- Major association with fried rice from Chinese restaurants
- Associated with food kept warm, not hot (buffets)

Pathogenesis: 2 possible toxins:

- **Emetic toxin: preformed fast** (1–6 hours), similar to *S. aureus* with vomiting and diarrhea; associated with **fried rice**
- Diarrheal toxin produced in vivo (meats, sauces): 18 hours, similar to *E. coli;* LT: increasing cAMP → watery diarrhea

Diseases

- Gastroenteritis: nonbloody, ± vomiting
- Eye infection (rare)

Key Vignette Clues

Bacillus cereus

- Rapid-onset gastroenteritis
- Fried rice, Chinese restaurants

Diagnosis

- Clinical grounds
- Culture and Gram stain of implicated food

Treatment: self-limiting; vancomycin for eye infection

GENUS: *CLOSTRIDIUM*

Species of Medical Importance

- *Clostridium tetani*
- *botulinum*
- *Clostridium perfringens*
- *Clostridium septicum*
- *Clostridium difficile*

Clostridium tetani

Distinguishing Features: large gram-positive, spore-forming rods; anaerobes; produces tetanus toxin

Reservoir: soil

Transmission: puncture wounds/trauma (human bites); requires low tissue oxygenation (E_h)

Key Vignette Clues

Clostridium tetani

- Dirty puncture wound
- Rigid paralysis

Pathogenesis

- Spores germinate in tissues, producing **tetanus toxin** (an exotoxin also called **tetanospasmin)**
- Carried intra-axonally to CNS
- **Binds to ganglioside receptors**
- **Blocks release of inhibitory mediators (glycine and GABA) at spinal synapses**
- Excitatory neurons are unopposed → extreme muscle spasm
- **One of the most toxic substances known**

Disease: tetanus

- Risus sardonicus
- Opisthotonus
- Extreme muscle spasms
- Drooling, hydrophobia

Diagnosis: primarily a clinical diagnosis; organism rarely isolated

Treatment of Actual Tetanus

- **Hyperimmune human globulin (TIG) to neutralize toxin plus metronidazole or penicillin**
- **Spasmolytic drugs** (diazepam); debride; delay closure

Prevention: toxoid is formaldehyde-inactivated toxin (important because disinfectants have poor sporicidal action); care of wounds: proper wound cleansing and care plus treatment

Table II-2-13. Wound Management

Patient	Not Tetanus Prone	Tetanus Prone
	Linear, 1 cm deep cut, without devitalized tissue, without major contaminants, <6 hours old	Blunt/missile, burn, frostbite, 1 cm deep; devitalized tissue present + contaminants (e.g., dirt, saliva); any wound 6 hours old
Not completed primary or vaccination history unknown	Vaccine	Vaccine and tetanus immunoglobulin (human)
Completed primary series	Vaccine if >10 years since last booster	Vaccine if >5 years since last booster

Clostridium botulinum

Distinguishing Features

- **Anaerobic**
- **Gram-positive spore-forming rods**

Reservoir: soil/dust

Transmission: foodborne/traumatic implantation

Pathogenesis

- **Spores** survive in soil and dust; **germinate** in moist, warm, nutritious but **nonacidic and anaerobic conditions**
- **Botulinum toxin**
  - **A-B polypeptide neurotoxin** (actually a series of 7 antigenically different; type A and B most common)
  - **Coded for by a prophage** (lysogenized *Clostridium botulinum*).
  - Highly toxic

Key Vignette Clues

Clostridium botulinum

- Home-canned alkaline vegetables
- Floppy baby syndrome (infant with flaccid paralysis)
- Reversible flaccid paralysis

– **Heat labile** (unlike *Staph*), 10 minutes 60.0°C

– **Mechanism of action: absorbed by gut** and carried by blood to peripheral nerve synapses; **blocks release of acetylcholine** at myoneural junction, resulting in a reversible **flaccid paralysis**

Disease(s)

Table II-2-14. Forms of Botulism

Disease	Adult	Infant
Acquisition	**Preformed toxin ingested (toxicosis)** Poorly canned alkaline vegetables (green beans)	**Spores ingested: household dust, honey** Toxin produced in gut (toxi-infection)
Symptoms	1–2 day onset of weakness, dizziness, blurred vision, flaccid paralysis (reversible), constipation	Constipation, limpness/flaccid paralysis (reversible): diplopia, dysphagia, weak feeding/crying; may lead to respiratory arrest
Toxin demonstrated in	Suspected food	Stool or serum
Treatment	Respiratory support Trivalent (A-B-E) antitoxin	Respiratory support in monitored intensive care; hyperimmune human serum Antibiotics generally not used as may worsen or prolong
Prevention	**Proper canning; heat all canned foods**	**No honey first 2 years**

Clostridium perfringens

Distinguishing Features

- Large **gram-positive, spore-forming** rods (spores rare in tissue), nonmotile
- **Anaerobic: "stormy fermentation" in milk media**
- **Double zone of hemolysis**

Reservoir: soil and human colon

Transmission: **foodborne and traumatic implantation**

Key Vignette Clues

Clostridium perfringens

- Contaminated wound
- Pain, edema, gas, fever, tachycardia
- Food poisoning: reheated meats, noninflammatory diarrhea

Pathogenesis

- **Spores** germinate under anaerobic conditions in tissue.
- Vegetative cells produce **alpha toxin** (phospholipase C), a **lecithinase**, which disrupts membranes, damaging RBCs, platelets, WBCs, and endothelial cells. Can lead to massive hemolysis, tissue destruction, and hepatic toxicity.
- Identified by **Nagler reaction:** egg yolk agar plate: one side with anti-α-**toxin**; lecithinase activity is detected on side with no antitoxin
- Twelve other toxins damage tissues
- **Enterotoxin** produced in intestines in food poisoning: disrupts ion transport → watery diarrhea, cramps (similar to *E. coli*); resolution <24 hours.

Disease(s)

- Gas gangrene (myonecrosis)
  - Contamination of **wound with soil or feces**
  - **Acute** and **increasing pain** at wound site
  - **Tense tissue** (edema, gas) and exudate
  - Systemic symptoms include **fever** and **tachycardia** (disproportionate to fever), diaphoresis, pallor, etc.
  - **Rapid, high mortality**
- Food poisoning: **reheated meat dishes,** organism grows to high numbers; 8–24 hour incubation; **enterotoxin** production in gut; self-limiting noninflammatory, watery diarrhea

Diagnosis: clinical

Treatment: debridement, delayed closure, clindamycin and penicillin, hyperbaric chamber for gangrene; food poisoning is self-limiting

Prevention: extensive **debridement** of wound plus penicillin

Clostridium septicum

Distinguishing features: anaerobic, gram-positive rods

Transmission: endogenous

Disease: septic shock in colon cancer patients

Clostridium difficile

Reservoir: human colon/gastrointestinal tract

Transmission: endogenous

Key Vignette Clues

Clostridium difficile

- Hospitalized patient on antibiotics
- Develops colitis, diarrhea

Pathogenesis

- **Toxin A: enterotoxin damaging mucosa leading to fluid increase; granulocyte attractant**
- **Toxin B: cytotoxin: cytopathic**

Disease(s): antibiotic-associated (clindamycin, cephalosporins, amoxicillin, ampicillin) **diarrhea, colitis, or pseudomembranous colitis** (yellow plaques on colon)

Diagnosis: culture is not diagnostic because organism is part of normal flora; stool exam for toxin production

Treatment

- **Metronidazole** for severe disease; vancomycin only when no other drug is available to avoid selecting for vancomycin-resistant normal flora
- Fecal transplant for chronic infections
- Discontinuation of other antibiotic therapy for mild disease

Prevention: use caution in overprescribing broad-spectrum antibiotics (consider limited-spectrum drugs first); in nursing home setting, isolate patients who are symptomatic; use autoclave bed pans (treatment kills spores)

GENUS: *LISTERIA*

Listeria monocytogenes

Distinguishing Features

- Small gram-positive rods
- Beta hemolytic, nonspore-forming rod on blood agar, CAMP positive
- **Tumbling motility** in broth; actin jet motility in cells
- **Facultative intracellular parasite**
- **Cold growth**

Reservoir

- Widespread: animals (GI and genital tracts), **unpasteurized milk products**, plants, and soil
- Cold growth: contaminated food, soft cheese, deli meat, cabbage (coleslaw), hot dogs, fruit, ice cream

Transmission: foodborne or vertical

Pathogenesis

- **Listeriolysin O, a β-hemolysin**, facilitates rapid egress from phagosome into cytoplasm, thus evading killing when lysosomal contents are dumped into phagosome; “jets” directly (by actin filament formation) from cytoplasm to another cell
- **Immunocompromised status** predisposes to serious infection

Key Vignette Clues

Listeria monocytogenes

- Gram (+), β hemolytic bacilli, cold growth
- Facultative intracellular
- Foodborne (deli foods)
- Transplacental granulomatosis infantiseptica
- Neonatal septicemia and meningitis (third most common cause)
- Meningitis in renal transplant or cancer patients (most common cause)

Disease(s)

- Listeriosis (human, peaks in summer)
  - Healthy adults and children: generally asymptomatic or diarrhea with low % carriage
  - Pregnant women: symptomatic carriage, septicemia characterized by fever and chills; can cross the placenta in septicemia.
- Neonatal disease
  - **Early-onset:** (granulomatosis infantisepticum) in utero transmission; sepsis with high mortality; disseminated granulomas with central necrosis
  - **Late-onset:** 2–3 weeks after birth from fecal exposure; meningitis with septicemia
- In immunocompromised patients
  - **Septicemia and meningitis** (most common clinical presentation)
  - *Listeria* meningitis most common cause of meningitis in **renal transplant patients and adults with cancer**

Diagnosis: blood or CSF culture at 4˚C; CSF or Gram stain (>25% lymphocytes + PMNs)

Treatment: ampicillin with gentamicin added for immunocompromised patients

Prevention: pregnant and immunocompromised patients should avoid cold deli food

GENUS: CORYNEBACTERIUM

Corynebacterium diphtheriae

Distinguishing Features

- Gray-to-black colonies of **club-shaped** gram-positive rods arranged in V or L shapes on **Gram stain**
- **Granules (volutin)** produced on Loeffler coagulated serum medium stain metachromatically
- **Toxin-producing strains have** β**-prophage** carrying genes for the toxin (**lysogeny,** β**-corynephage**). The phage from one person with diphtheria can infect the normal nontoxigenic diphtheroid of another, and thus cause diphtheria.

Reservoir: throat and nasopharynx

Transmission: bacterium or phage via respiratory droplets

Key Vignette Clues

Corynebacterium diphtheriae

- Gram (+), aerobic, non–spore forming rods
- Bull neck, myocarditis, nerve palsies
- Gray pseudomembrane → airway obstruction
- Toxin produced by lysogeny
- Toxin ribosylates eEF-2; heart, nerve damage

Pathogenesis

- Organism **not invasive**; colonizes epithelium of oropharynx or skin in cutaneous diphtheria
- **Diphtheria toxin (A-B component)—inhibits protein synthesis by adding ADP-ribose to eEF-2**
- Effect on oropharynx: **Dirty gray pseudomembrane** (made up of dead cells and fibrin exudate, bacterial pigment)
- **Extension into larynx/trachea → obstruction**
- Effect of systemic circulation → **heart** and **nerve** damage

Disease: diphtheria (sore throat with **pseudomembrane, bull neck**, potential respiratory obstruction, **myocarditis**, cardiac dysfunction, **recurrent laryngeal nerve palsy**, and lower limb polyneuritis), renal failure

Diagnosis

- Elek test to document toxin production (ELISA for toxin is now gold standard)
- Toxin produced by Elek test toxin-producing strains diffuses away from growth
- Antitoxin diffuses away from strip of filter paper
- Precipitin lines form at zone of equivalence

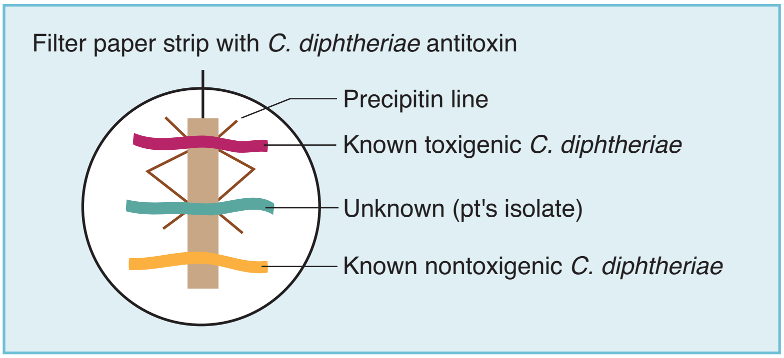


Figure II-2-2. Elek Test

Treatment

- Erythromycin and antitoxin
- For endocarditis, intravenous penicillin and aminoglycosides for 4–6 weeks

Prevention: toxoid vaccine (formaldehyde-modified toxin is still immunogenic but with reduced toxicity), part of DTaP, DTP, or Td, boosters 10-year intervals

GENUS: *ACTINOMYCES*

Actinomyces israelii

Distinguishing Features

- Anaerobic
- Branching rods
- Non–acid fast

Reservoir: human; normal flora of **gingival crevices** and **female genital tract**

Transmission: endogenous

Pathogenesis: invasive growth in tissues with compromised oxygen supply

Disease: actinomycosis

- Generally not painful but **very invasive**, penetrating all tissues including bone
- **Tissue swelling** can lead to **draining abscesses** (sinus tracts) with **"sulfur" granules** (hard yellow microcolonies) in exudate that can be used for microscopy or culture
- Only in tissues with low oxygenation (E_h)
  - **Cervicofacial (lumpy jaw): dental trauma or poor oral hygiene**
  - **Pelvic: from thoracic or sometimes IUDs**
  - Abdominal: surgery or bowel trauma
  - Thoracic: aspiration with contiguous spread
  - CNS: **solitary brain abscess** (*Nocardia* will produce multiple foci)

Diagnosis: identify gram-positive branching bacilli in "sulfur granules"; colonies resemble molar tooth

Treatment: penicillin V and surgical drainage; metronidazole not effective

Key Vignette Clues

- Patient with mycetoma on jaw line or spread from IUD
- Sulfur granules in pus grow anaerobic, gram (+), non-acid fast branching rods

GENUS: *NOCARDIA*

Nocardia asteroides and *Nocardia brasiliensis*

Distinguishing Features: aerobic; Gram-positive branching rods (some areas of smear will be blue and some red; **partially acid fast)**

Reservoir: soil and dust

Transmission: airborne or traumatic transplantation

Pathogenesis: no known toxins or virulence factors; **immunosuppression** and **cancer predispose** to pulmonary infection

Key Vignette Clues

Nocardia asteroides* and *Nocardia brasiliensis

- Gram (+) filamentous bacilli, aerobic, partially acid fast
- Cavitary bronchopulmonary disease, mycetomas

Disease(s)

- Nocardiosis
  - **Cavitary bronchopulmonary nocardiosis**
  - Mostly *N. asteroides*
  - Can be acute, subacute, chronic
  - Symptoms: cough, fever, dyspnea, localized or diffuse pneumonia with cavitation
  - May spread hematogenously to brain (**brain abscesses**)
- Cutaneous/subcutaneous nocardiosis
  - Mostly *N. brasiliensis*
  - Starts with traumatic implantation
  - Symptoms: **cellulitis** with swelling can lead to **draining subcutaneous abscesses** with **granules** (mycetoma)

Diagnosis: culture of sputum or pus from cutaneous lesion

Treatment: sulfonamides (high dose) or trimethoprim/sulfamethoxazole (TMP-SMX)

GENUS: *MYCOBACTERIUM*

Genus Features

- **Acid fast rods** with waxy cell wall
- **Obligate aerobe**
- Cell wall
  - Unique: **high concentration of lipids** containing long chain fatty acids called **mycolic acids**
  - Wall makes **mycobacteria highly resistant to desiccation** and **many chemicals** (including NaOH used to kill other bacteria in sputa before neutralizing and culturing)
- **Sensitive to UV**

Species of Medical Importance

- *M. tuberculosis*
- *M. leprae*
- *M. avium-intracellulare*
- *M. kansasii*
- *M. marinum*
- *M. ulcerans*

Mycobacterium tuberculosis

Distinguishing Features

- **Auramine-rhodamine staining bacilli** (fluorescent apple green); no antibody involved (sensitive but not specific)
- **Acid fast**
- **Aerobic, slow growing** on **Lowenstein-Jensen** medium; new culture systems (broths with palmitic acid) faster
- **Produces niacin**
- **Produces a heat-sensitive catalase**: catalase-negative at 68.0°C (154.4 F) (standard catalase test); catalase active at body temperature

Reservoir: human lungs

Transmission: respiratory droplets

Pathogenesis

- **Facultative intracellular organism** (most important)
- **Sulfatides (sulfolipids** in cell envelope): **inhibit phagosome-lysosome fusion,** allowing intracellular survival (if fusion occurs, waxy nature of cell envelope reduces killing effect)
- **Cord factor (trehalose dimycolate):** causes **serpentine growth** in vitro; **inhibits leukocyte migration; disrupts mitochondrial respiration and oxidative phosphorylation**
- **Tuberculin** (surface protein) along with mycolic acid → delayed hypersensitivity and **cell-mediated immunity** (CMI): granulomas and caseation mediated by CMI; no exotoxins or endotoxin; damage done by immune system

Disease(s)

- Primary pulmonary TB
  - Symptoms can include fever, dry cough
  - Organisms replicate in naive alveolar macrophages, killing the macrophages until CMI is set up (Ghon focus)
  - Macrophages transport the bacilli to the regional lymph node (**Ghon complex**) and most people heal without disease
  - Organisms that are walled off within the Ghon complex remain viable unless treated
- Reactivational TB
  - Symptoms can include fever, hemoptysis, night sweats, weight loss
  - Erosion of granulomas into airways (high oxygen) later in life under conditions of reduced T-cell immunity can lead to mycobacterial replication and disease symptoms

Key Vignette Clues

Mycobacterium tuberculosis

- High-risk patient (poverty, HIV+, IV drug user)
- Chronic cough, weight loss
- Ghon complex
- Auramine-rhodamine staining, acid fast bacilli in sputum
- Produce niacin, heat-sensitive catalase
- Positive PPD
- Facultative intracellular

Cord Factor

- Complex disease with the potential of infecting any organ system
- May disseminate (miliary TB): kidneys, GI tract, brain, spine (Pott disease)

Diagnosis

- Microscopy of sputum: screen with auramine-rhodamine stain (fluorescent apple-green); no antibody involved; very sensitive; if positive, confirm with
- acid fast stain
- **PPD skin test** (Mantoux): measure **zone of induration at 48–72 hours; positive if:**
  - ≥5 mm in HIV+ or anyone with recent TB exposure; AIDS patients have reduced ability to mount skin test.
  - ≥10 mm in high-risk population: IV drug abusers, people living in poverty, or immigrants from high TB area
  - ≥15 mm in low-risk population
- **Positive skin test indicates** only **exposure but not necessarily active disease.**
- Quantiferon-TB Gold Test: measures interferon-gamma production when leukocytes exposed to TB antigens
- Slow-growing (3–6 weeks) colonies on Lowenstein-Jensen medium (faster new systems)
- Organisms produce **niacin** and are **catalase-negative** (68°C).
- **No serodiagnosis**

Treatment

- **Multiple drugs critical** to treat infection
- Standard observed short-term therapy for uncomplicated pulmonary TB (rate where acquired resistance <4%):
  - First 2 months: rifampin + isoniazid + pyrazinamide + ethambutol (RIPE)
  - Next 4 months: rifampin and isoniazid
- Streptomycin added for possible drug-resistant cases until susceptibility tests are back (if area acquired has >4% drug-resistant mycobacteria)
- For MDR TB, use 3–5 previously unused drugs: aminoglycosides, fluoroquinolones, thioamide, cycloserine, bedaquiline

Prevention

- **Isoniazid** taken for 9 months can prevent TB in persons with infection but no clinical symptoms
- Bacille Calmette-Guérin (BCG) **vaccine** containing live, attenuated organisms may prevent disseminated disease; not used in U.S.
- UV light or HEPA filter used to treat potentially contaminated air

Mycobacteria Other than Tuberculosis (MOTTS)

Distinguishing Features

- Atypical mycobacteria
- **Noncontagious**
- Found in surface waters, soil, cigarettes

Note

- *M. tuberculosis* in HIV patient with normal count or low CD4 count (disseminated)
- MAI only in late HIV patient with low CD4 count

Table II-2-15. MOTTS

Organism	Disease	Transmission	Clinical Presentation	Treatment
M. avium-intracellulare	Pulmonary, GI, Disseminated	Respiratory, Ingestion	AIDS patients, cancer, chronic lung disease	AIDS patients prophylaxis <50 CD4+ cells/mm^3 Macrolide plus ethambutol
M. kansasii				
M. ulcerans	Buruli ulcer (Africa)	Wound contamination	Ulcer	Rifampin and streptomycin
M. marinum	Soft tissue infections "Fish tank granuloma"	Abrasions	Cutaneous granulomas in tropical fish enthusiasts	Isoniazid Rifampin or ethambutol

Mycobacterium leprae

Distinguishing Features

- **Acid fast rods (seen in punch biopsy)**
- **Obligate intracellular parasite** (cannot be cultured in vitro)
- Optimal growth at less than body temperature

Reservoir: human mucosa, skin, and nerves are only significant reservoirs; some infected armadillos in Texas and Louisiana

Transmission: nasal discharge from untreated lepromatous leprosy patients

Pathogenesis: obligate intracellular parasite; cooler parts of body, e.g., skin, mucous membranes, and peripheral nerves

Disease(s): leprosy: a continuum of disease, which usually starts out with an indeterminate stage called "borderline"

Key Vignette Clues

Leprosy

- Acid fast bacilli in punch biopsy
- Immigrant patient with sensory loss in extremities

Table II-2-16. Extreme Forms of Leprosy

	Tuberculoid	Borderline	Lepromatous
Cell-mediated immune system	Strong CMI (Th1)		Weak CMI (Th2)
Lepromin skin test	Lepromin test +		Lepromin test –
Number of organisms in tissue	Low		High (foam cells totally filled)
Damage from	• Immune response (CMI killing infected cells) • Granuloma formation → nerve enlargement/damage • Loss of sensation → burns and trauma		• Large number of intracellular organisms • Nerve damage from overgrowth of bacteria in cells • Loss of sensation → burns and trauma
Number of lesions and other symptoms	Fewer lesions: macular; nerve enlargement, paresthesia		• Numerous lesions becoming nodular; loss of eyebrows; destruction of nasal septum • Paresthesia • Leonine facies

Diagnosis

- **Punch biopsy or nasal scrapings; acid fast stain**
- **Lepromin skin test** is positive in the tuberculoid but not in the lepromatous form
- No cultures

Treatment: multiple-drug therapy with dapsone and rifampin, with clofazimine added for lepromatous

Prevention: dapsone for close family contacts

GRAM-NEGATIVE COCCI

GENUS: *NEISSERIA*

Table II-2-17. Medically Important *Neisseria* Species

Organism	Capsule	Vaccine	Portal of Entry	Glucose Fermentation	Maltose Fermentation	β-Lactamase Production
N. meningitidis	Yes	Yes	Respiratory	Yes	Yes	Rare
N. gonorrhoeae	No	No	Genital	Yes	No	Common

Neisseria meningitidis

Distinguishing Features

- **Gram-negative, kidney bean–shaped diplococci**
- **Oxidase-positive**
- **Large capsule; latex particle agglutination** (or CIE, counter immuno-electrophoresis) **to identify *N. meningitidis* capsular antigens in CSF**
- Grows on **chocolate (not blood) agar in 5% CO_2** atmosphere
- **Ferments maltose** in contrast to gonococci

Reservoir: human nasopharynx (5–10% carriers)

Transmission: respiratory droplets; oropharyngeal colonization, spreads to meninges via bloodstream; disease occurs in only small percentage of colonized individuals

Pathogenesis: important virulence factors

- Polysaccharide **capsule:** antiphagocytic, antigenic, 5 common serogroups: B is not strongly immunogenic (sialic acid); B strain is most common strain in U.S.; used for serogrouping, detection in CSF, and vaccine
- **IgA protease** allows oropharynx colonization.
- **Endotoxin** (lipo**oligo**saccharide): fever, septic shock in meningococcemia, **overproduction of outer membrane**
- Pili and outer membrane proteins important in ability to colonize and invade
- Deficiency in late complement components (C5–C9) predisposes to bacteremia

Note

Oxidase

- *Oxidase (cytochrome C oxidase) test:* flood colony with phenylenediamine; in presence of oxidase, phenylenediamine turns black. Rapid test.
- Major oxidase-negative gram-negative group is Enterobacteriaceae.

Key Vignette Clues

Meningococcal Meningitis

- Gram (–) diplococcus in CSF
- Young adults with meningitis
- Abrupt onset with signs of endotoxin toxicity

Disease(s): meningitis and meningococcemia

- **Abrupt onset with fever, chills, malaise, prostration, and rash that is generally petechial;** rapid decline
- Fulminant cases: ecchymoses, DIC, shock, coma, and death (Waterhouse-Friderichsen syndrome)

Diagnosis: Gram stain of CSF; PCR; latex agglutination

Treatment: ampicillin and cefotaxime (neonates/infants); cefotaxime or ceftriaxone with or without vancomycin (children and adults)

Prevention: vaccine: conjugate for strains **Y, W-135, C,** and **A,** recombinant for serotype **B,** prophylaxis of close contacts with **rifampin** (or ciprofloxacin)

Key Vignette Clues

Neisseria gonorrhoeae

- Sexually active patient
- Urethral/vaginal discharge (leukorrhea)
- Arthritis possible
- Neonatal ophthalmia
- Gram (–) diplococcus in neutrophils

Neisseria gonorrhoeae

Distinguishing Features

- **Gram-negative**, kidney bean–shaped **diplococci**
- Oxidase-positive

Reservoir: human genital tract

Transmission: sexual contact, birth; sensitive to drying and cold

Pathogenesis

- **Pili: attachment** to mucosal surfaces; **inhibit phagocytic uptake**; **antigenic variation:** >1 million variants
- **Outer membrane proteins:** OMP I structural, antigen used in serotyping; Opa proteins (opacity) **antigenic variation,** adherence; **IgA protease** aids in colonization and cellular uptake
- **Organism invades mucosal surfaces and causes inflammation.**

Disease: gonorrhea

- Male: **urethritis,** proctitis
- Female: **endocervicitis,** PID (contiguous spread), arthritis, proctitis
- Infants: **ophthalmia**(rapidly leads to **blindness if untreated)**
- Disseminated disease: arthritis, skin lesions
- Gonococcal pharyngitis

Diagnosis

- Intracellular **gram-negative diplococci in PMNs** from urethral smear
- Commonly: diagnosis by **genetic probes** with amplification
- Culture (when done) on **Thayer-Martin medium**
  - Oxidase-positive colonies
  - Maltose not fermented
  - No capsule

Treatment: ceftriaxone; test for *C. trachomatis* or treat with macrolide (doxycycline alternative); plasmid-mediated β-**lactamase** produces **high-level penicillin resistance**

Prevention: condoms (adult forms have no vaccine); for neonates, silver nitrate or erythromycin ointment in eyes at birth

Moraxella catarrhalis

Distinguishing Features

- Gram-negative diplococcus
- Close relative of *Neisseria*

Reservoir: normal upper respiratory tract flora

Transmission: respiratory droplets

Pathogenesis: endotoxin may play role in disease

Disease(s): otitis media; sinusitis; bronchitis and bronchopneumonia in elderly patients with COPD

Treatment: amoxicillin and clavulanate, second- or third-generation cephalosporin or TMP-SMX; drug resistance is a problem (most strains produce a β-lactamase)

GRAM-NEGATIVE BACILLI

GENUS: *PSEUDOMONAS*

Genus Features

- **Gram-negative rod**
- **Oxidase-positive**, catalase-positive
- **Aerobic (nonfermenting)**

Species of Medical Importance: *Pseudomonas aeruginosa*

Key Vignette Clues

Pseudomonas

- Gram (–), oxidase (+), aerobic bacillus
- Blue-green pigments, fruity odor
- Burn infections—blue-green pus, fruity odor
- Typical pneumonia—CGD or CF
- UTI—catheterized patients

Pseudomonas aeruginosa

Distinguishing Features

- **Oxidase-positive, Gram-negative rods, nonfermenting**
- **Pigments: pyocyanin (blue-green)** and fluorescein
- **Grape-like odor**
- **Slime layer**
- **Non–lactose fermenting** colonies on EMB or MacConkey
- **Biofilm**

Reservoir: ubiquitous in water

Transmission: water aerosols, raw vegetables, flowers

Pathogenesis

- **Endotoxin** causes inflammation in tissues and gram-negative shock in septicemia
- Pseudomonas **exotoxin A ADP ribosylates eEF-2,** inhibiting protein synthesis (like diphtheria toxin)
- **Liver is primary target**
- **Capsule/slime layer** allows formation of pulmonary **microcolonies**; difficult to remove by phagocytosis

Disease(s)

- Healthy people: transient GI tract colonization (loose stool 10% population); hot tub folliculitis; eye ulcer (trauma, coma, prolonged contact wear)
- Burn patients: GI tract colonization → skin → colonization of eschar → **cellulitis (blue-green pus)** → **septicemia**
- Neutropenic patients: **pneumonia** and **septicemia** (often **superinfection,** i.e., infection while on antibiotics)
- Chronic granulomatous disease: pneumonias, septicemias (*Pseudomonas* is catalase-positive); [diabetic] osteomyelitis (diabetic foot)
- Otitis externa: swimmers, diabetics, those with pierced ears
- Septicemias: fever, shock ± skin lesions (black, necrotic center, erythematous margin, **ecthyma gangrenosum)**
- Catheterized patients: urinary tract infection
- Cystic fibrosis: early pulmonary colonization, recurrent pneumonia; **always** high **slime-producing strain**

Note

Pseudomonas Medical Ecology

Pseudomonas aeruginosa is a **ubiquitous water** and soil organism that grows to very high numbers overnight in standing water (distilled or tap). Sources for infection include:

- Raw vegetables, respirators, humidifiers, sink drains, faucet aerators, cut and potted flowers, and, if not properly maintained, whirlpools
- Transient colonization of colons in about 10% of people; bacteria get on skin from fecal organisms; requires exquisitely careful housekeeping and restricted diets in burn units

Diagnosis: Gram stain and culture

Treatment: antipseudomonal penicillin and an aminoglycoside or fluoroquinolone

Prevention: pasteurize or disinfect water-related equipment, hand washing; prompt removal of catheters; avoid flowers and raw vegetables in burn units

Burkholderia cepacia

Distinguishing Features: oxidase-positive, catalase-positive; non-fermenting

Transmission: water aerosols, person-person (respiratory droplets)

Disease(s): cystic fibrosis (recurrent pneumonia); CGD (pneumonia, septicemia)

Treatment: trimethoprim-sulfamethoxazole

Acinetobacter baumannii

Distinguishing Features: oxidase-negative; non-fermenting; biofilm

Transmission: wound infection or nosocomial

Disease(s): wound infection and pneumonia in military personnel; 'Iraqibacter'

Treatment: highly drug-resistant; carbapenem or polymyxin

GENUS: *LEGIONELLA*

Legionella pneumophila

Distinguishing Features

- **Stain poorly** with standard Gram stain; **gram-negative**
- **Fastidious** requiring increased **iron and cysteine** for laboratory culture (BCYE, buffered charcoal, yeast extract)
- **Facultative intracellular**

Reservoir: rivers/streams/amebae; air-conditioning water cooling tanks

Transmission: aerosols from contaminated **air-conditioning; no human-to-human transmission**

Predisposing Factors: smokers age >55 with **high alcohol** intake; **immunosuppressed patients** such as renal transplant patients

Pathogenesis: facultative intracellular pathogen; **endotoxin**

Note

Drug Resistance in *P. aeruginosa*

Susceptibilities important and drug resistance very common.

Intrinsic resistance (missing high affinity porin some drugs enter through); plasmid-mediated

β-lactamases and acetylating enzymes.

Key Vignette Clues

Legionella pneumophila

- Elderly smoker, heavy drinker, or Immunosuppressed
- Exposure to aerosols of water
- Atypical pneumonia
- Triad of atypical pneumonia, diarrhea, and hyponatremia

Disease(s)

- Legionnaires disease ("atypical pneumonia"): associated with air-conditioning systems (now routinely decontaminated); pneumonia; hyponatremia; mental confusion; diarrhea (no *Legionella* in GI tract)
- Pontiac fever: pneumonitis; no fatalities

Diagnosis

- **Urinary antigen test (serogroup 1)**
- **DFA** (direct fluorescent antibody) on biopsy, (+) by Dieterle silver stain
- Fourfold increase in antibody

Treatment: fluoroquinolone (levofloxacin) or macrolide (azithromycin) with rifampin (immunocompromised patients); drug must penetrate human cells.

Prevention: routine decontamination of air-conditioner cooling tanks

GENUS: *FRANCISELLA*

Key Vignette Clues

Francisella tularensis

- Hunter with ulceroglandular disease, atypical pneumonia, or GI disease
- Arkansas/Missouri
- Exposure to rabbits, ticks

Francisella tularensis

Distinguishing Features

- Small gram-negative rod
- **Potential biowarfare agent**
- **Zoonosis**

Reservoir: many species of wild **animals**, especially rabbits, deer, and rodents; endemic in every state of the U.S. but highest in Arkansas and Missouri

Transmission

- **Tick bite** *(Dermacentor)* → **ulceroglandular** disease, characterized by **fever**, ulcer at bite site, and regional lymph node enlargement and necrosis
- **Traumatic implantation** while skinning rabbits → ulceroglandular disease
- Aerosols (skinning rabbits) → pneumonia
- Ingestion (of undercooked, infected meat or contaminated water) produces typhoidal tularemia.

Pathogenesis: facultative intracellular pathogen (localizes in reticuloendothelial cells); granulomatous response

Disease: ulceroglandular tularemia (open wound contact with rabbit blood; tick bite); pneumonic tularemia (bioterrorism; atypical pneumonia)

Diagnosis: serodiagnosis (culture is hazardous); DFA; grows on BCYE

Treatment: streptomycin

Prevention: protection against tick bites; glove use while butchering rabbits; live, attenuated vaccine (for those at high risk)

GENUS: *BORDETELLA*

Genus Features

- **Gram-negative small rods**
- **Strict aerobes**

Species of Medical Importance: *Bordetella pertussis*

Bordetella pertussis

Distinguishing Features: small **gram-negative,** aerobic **rods**; encapsulated organism

Reservoir: **human** (vaccinated)

Transmission: respiratory droplets

Pathogenesis

- ***B. pertussis* is mucosal surface pathogen**
- **Attachment** to nasopharyngeal ciliated epithelial cells is via filamentous hemagglutinin; pertussis toxin (on outer membrane) aids in attachment
- **Toxins** damage respiratory epithelium.
  - **Adenylate cyclase toxin:** impairs leukocyte chemotaxis → inhibits phagocytosis and causes local edema
  - **Tracheal cytotoxin:** interferes with ciliary action; kills ciliated cells
  - Endotoxin
  - **Pertussis toxin** (A and B component, OM protein toxin): **ADP ribosylation of G_i** (inhibiting negative regulator of adenylate cyclase) interferes with transfer of signals from cell surface to intracellular mediator system: lymphocytosis; islet-activation leading to hypoglycemia; blocking of immune effector cells (decreased chemotaxis); increased histamine sensitivity

Key Vignette Clues

Bordetella pertussis

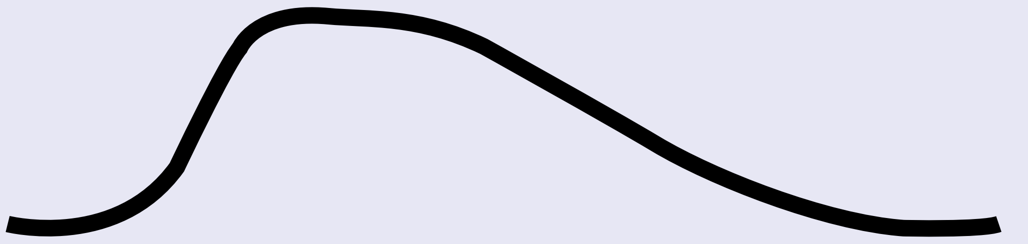
- Unvaccinated child (immigrant family or religious objections)
- Cough with inspiratory "whoop"

Note

B. pertussis Immunity

- Vaccine immunity lasts 5–10 yrs (and is primarily IgA)
- Babies born with little immunity
- Vaccinated humans >10 yrs serve as reservoir
- 12–20% of afebrile adults with cough >2 wks have pertussis
- Vaccine (DTaP)
- Acellular
- Components: immunogens vary by manufacturer; pertussis toxoid; filamentous hemagglutinin; pertactin (OMP); one other

Table II-2-18. Stages of Whooping Cough (Pertussis) vs. Results of Bacterial Culture

	Incubation	Catarrhal	Paroxysmal	Convalescent
Duration	7–10 days	1–2 weeks	2–4 weeks	3–4 weeks (or longer)
Symptoms	None	Rhinorrhea, malaise, sneezing, anorexia	Repetitive cough with whoops, vomiting, leukocytosis	Diminished paroxysmal cough, development of secondary complications (pneumonia, seizures, encephalopathy)
Bacterial Culture				

Diagnosis

- Fastidious/delicate: **Regan-Lowe** or Bordet-Gengou media; either direct cough plates or nasopharyngeal cultures
- Difficult to culture from middle of paroxysmal stage on
- Direct immunofluorescence **(DFA)** on nasopharyngeal smear
- PCR and serologic tests available

Treatment: supportive care, i.e., hospitalization if age <6 months; erythromycin for 14 days including all household contacts

Prevention: vaccine DTaP (acellular pertussis: filamentous hemagglutinin plus pertussis toxoid); immunity wanes 5–7 years; babies are born with little or no immunity (IgA) from mother

GENUS: *BRUCELLA*

Brucella Species

Distinguishing Features

- Small **gram-negative** rods, aerobic
- Facultative intracellular
- **Zoonosis**
- Culture is hazardous
- **Potential bioterrorism agent**

Reservoir: domestic livestock

Key Vignette Clues

Brucella

- Patient with acute septicemia
- Exposure to animals or unpasteurized dairy
- California/Texas or travel to Mexico

Transmission

- **Unpasteurized dairy products** (California and Texas highest number of cases; most associated with travel to Mexico)
- **Direct contact with the animal,** work in slaughterhouse
  - *Brucella abortus*: cattle
  - *Brucella melitensis:* goats
  - *Brucella suis:* pigs
  - *Brucella canis:* dogs

Pathogenesis

- **Endotoxin**
- **Facultative intracellular parasite** (localizes in cells of reticuloendothelial system) → **septicemia**
- **Granulomatous response** with central necrosis

Disease: brucellosis (undulant fever)

- Acute septicemias
- Fever 37.7–40.0 C (100.0–104.0 F) (often in evening)
- Influenza-like symptoms, including arthralgias, anorexia, myalgia, back pain
- **Profuse sweating**
- Hepatomegaly

Diagnosis: culture is hazardous; serum agglutination test, fourfold increase in titer; antibodies against *Brucella* >1:160 considered positive

Treatment: rifampin and doxycycline minimum 6 weeks (adults); rifampin and cotrimoxazole (children)

Prevention: vaccinate cattle; pasteurize milk (especially goat milk)

GENUS: *HAEMOPHILUS*

Haemophilus influenzae

Distinguishing Features

- Encapsulated, **gram-negative rod; 95%** of invasive disease caused by capsular type b
- **Requires growth factors X (hemin) and V (NAD) for growth** on nutrient or blood agar (BA)
- Grows near ***S. aureus*** **on BA = "satellite" phenomenon**
- Chocolate agar provides both X and V factors

Note

Zoonotic Organisms

- *Brucella*
- *Bacillus anthracis*
- *Listeria monocytogenes*
- *Salmonella enteritidis*
- *Campylobacter*
- *Chlamydophila psittaci*
- *Francisella tularensis*
- *Yersinia pestis*

Key Vignette Clues

Haemophilus influenzae

- Unvaccinated child 3 mo–2 y: meningitis, pneumonia, epiglottitis
- Smokers with COPD: bronchitis, pneumonia
- Gram (–) rod, requires factors X and V

Reservoir: human nasopharynx

Transmission: respiratory droplets, shared toys

Pathogenesis

- **Polysaccharide capsule (type b capsule is polyribitol phosphate)** most important virulence factor
- Capsule important in diagnosis; **antigen screen on CSF** (e.g., latex particle agglutination); serotype all isolates by quellung.
- **IgA protease** is a mucosal colonizing factor.

Diseases

- Meningitis
  - **Epidemic in unvaccinated children ages 3 months to 2 years**
  - After maternal antibody has waned and before immune response of child is adequate
  - Up to 1990, *H. influenzae* was most common cause of meningitis age 1–5 (mainly <2); is still a problem if child age <2 and not vaccinated
- Otitis media: usually nontypeable strains
- Bronchitis: exacerbations of acute bronchitis in smokers with COPD
- Pneumonia: 1–24 months; rare in vaccinated children; smokers
- Epiglottitis: rare in vaccinated children; seen in unvaccinated toddlers; ***H. influenzae*** **was major causal agent**

Diagnosis: blood or CSF culture on chocolate agar; PCR; antigen detection of capsule (latex particle agglutination)

Treatment: cefotaxime or ceftriaxone for empirical therapy of meningitis; check nasal carriage before releasing; use rifampin if still colonized

Prevention

- **Conjugate capsular polysaccharide-protein vaccine**
- **Vaccination effective** to prevent type b disease
  - **Polyribitol capsule conjugated to protein:** (diphtheria toxoid or *N. meningitidis* outer membrane proteins), making it a **T-cell dependent vaccine**
  - Vaccine: 2, 4, 6 months; booster 15 months; 95% effective
- Rifampin reduces oropharynx colonization and prevents meningitis in unvaccinated, close contacts age <2 years

Haemophilus ducreyi

Reservoir: human genitals

Transmission: sexual transmission and direct contact

Diseases

- Chancroid
- Genital ulcers: **soft, painful chancre** ("You do cry with ducreyi.")
- **Slow to heal without treatment**
- **Open lesions increase transmission of HIV.**

Diagnosis: DNA probe, 'school of fish' appearance on Gram stain

Treatment: azithromycin, ceftriaxone, or ciprofloxacin

GENUS: *PASTEURELLA*

Pasteurella multocida

Distinguishing Features: small gram-negative rods; facultative anaerobic rods

Reservoir: mouths of many animals, especially cats and dogs

Transmission: animal bites; **particularly from cat bites**

Pathogenesis: endotoxin, capsule; spreads rapidly within skin, no exotoxins known

Disease: cellulitis with lymphadenitis (rapidly spreading wound infections, frequently polymicrobial infections)

Diagnosis: rarely cultured because routine prophylaxis is common

Treatment: amoxicillin/clavulanate for cat bites; resistant to macrolides

Prevention: amoxicillin/clavulanate is standard prophylaxis and treatment for most bites (including human), along with thorough cleaning

Key Vignette Clues

Pasteurella multocida

- Patient with animal (cat) bite
- Cellulitis/lymphadenitis

Table II-2-19. Additional Organisms Associated with Animal/Human Bites

Organism	Characteristics	Reservoir/ Transmission	Disease	Treatment
Eikenella corrodens	Gram-negative rods **"corrodes" agar; bleach-like odor**	Human oropharynx **human bites or fist fight injuries**	Cellulitis	Third-generation cephalosporins; fluoroquinolones
Capnocytophaga canimorsus	Gram-negative filamentous rods	Dog oropharynx/**dog bite wounds**	Cellulitis splenectomy → overwhelming sepsis	Third-generation cephalosporins; fluoroquinolones resistant to aminoglycosides
Bartonella henselae	Gram-negative rods	Cats and dogs/ bites, scratches, fleas	Cat scratch fever; **bacillary angiomatosis (AIDS)**	Azithromycin; doxycycline

HACEK Group Infections

- Group of gram-negative fastidious rods
  - *Aggregatibacter aphrophilus* (formerly *Haemophilus aphrophilus*)
  - *Aggregatibacter actinomycetemcomitans*
  - *Cardiobacterium hominis*
  - *Eikenella corrodens*
  - *Kingella kingae*
- HACEK organisms responsible for 5–10% of infective endocarditis cases (usually subacute)
- Most common cause of gram-negative endocarditis in non–IV drug users
- All part of normal oral flora
- Difficult to diagnose, with mean diagnosis time 3 months
- Treat with third-generation cephalosporin or fluoroquinolone

GENUS: *CAMPYLOBACTER*

Key Vignette Clues

Campylobacter jejuni

- Patient with inflammatory diarrhea
- Gram (–), curved rod, microaerophilic, oxidase (+), grows at 42°C (107.6 F)

Campylobacter jejuni

Distinguishing Features: **Gram-negative curved rods with polar flagella (“gulls’ wings”);** oxidase-positive; microaerophilic; **grows at 42˚C** on Campy or Skirrow agar

Reservoir: intestinal tracts of humans, cattle, sheep, dogs, cats, **poultry**

Transmission: fecal-oral, Skirrow agar primarily from **poultry**

Pathogenesis: **low infectious dose (as few as 500); invades** mucosa of the colon, destroying mucosal surfaces; blood and **pus** in stools (inflammatory diarrhea); rarely penetrates to cause septicemia

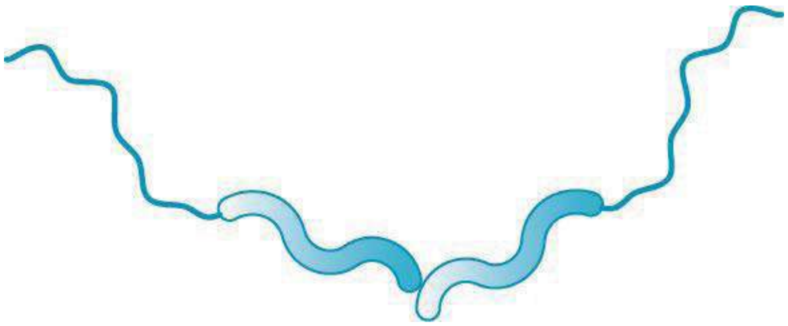


Figure II-2-3. Campylobacter

Disease: gastroenteritis

- Common cause of infectious diarrhea worldwide
- In U.S., *Campylobacter* enteritis > (*Salmonella* plus *Shigella*)
- **≥10 stools/day** (may be **frankly bloody**)
- Abdominal pain, fever, malaise, nausea, and vomiting
- Generally **self-limiting in 3–5 days** but may last longer
- Complications
  - **Guillain-Barré syndrome (GBS)** → 30% of GBS in U.S. Serotype O:19, antigenic cross-reactivity between *Campylobacter* oligosaccharides and glycosphingolipids on neural tissues
  - Reactive arthritis (HLA-B27)

Diagnosis: culture on *Campylobacter* or Skirrow agar at 42.0° C (107.6 F); microaerophilic

Treatment: mostly supportive, i.e., fluid and electrolyte replacement; erythromycin, fluoroquinolones; resistant to penicillins

GENUS: *HELICOBACTER*

Helicobacter pylori

Distinguishing Features: Gram-negative spiral gastric bacteria with flagella; oxidase-positive, urease-positive; microaerophilic, grows at 37°C on Campy or Skirrow agar

Reservoir: humans

Transmission: fecal-oral; oral-oral

Pathogenesis

- **Motile**
- **Urease-positive:** ammonium cloud neutralizes stomach acid, allowing survival in stomach acid during transit to border
- **Mucinase** aids in penetration of mucous layer (rapid shift down to neutral as it penetrates)
- **Invasive** into stomach lining where pH is neutral
- Inflammation is prominent
- Two biotypes (I and II); type I produces vacuolating cytotoxin

Diseases: chronic gastritis and duodenal ulcers

- Associated with several forms of **stomach cancer** (gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue lymphoma [MALToma], B-cell lymphomas)
- Now classed by WHO as **type I carcinogen**

Key Vignette Clues

Helicobacter pylori

- Patient with gastritis, ulcers, stomach cancer
- Gram (–), helical bacilli, oxidase (+), microaerophilic, urease (+)

Diagnosis

- Biopsy with culture; histology with Giemsa or silver stain
- Urea breath test: ^{13}C-urea swallowed; ammonia+^{13}C-CO_2 exhaled
- Serology

Treatment: myriad of regimens

- Omeprazole + amoxicillin + clarithromycin is one example of triple therapy
- Treat for 10–14 days
- Quadruple therapy is used in areas where clarithromycin resistance is ≥15%, e.g., PPI + bismuth + 2 antibiotics (metronidazole + tetracycline)

GENUS: *VIBRIO*

Genus Features

- **Gram-negative curved rod with polar flagella**
- Oxidase positive
- Vibrionaceae
- Growth on **alkaline,** but not acidic, media (**TCBS, t**hiosulfate **c**itrate **b**ile salt **s**ucrose medium)

Species of Medical Importance

- *Vibrio cholerae*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*

Vibrio cholerae

Distinguishing Features: rice-water diarrhea; growth on TCBS

Reservoir

- Human colon; **no vertebrate animal carriers** (copepods or shellfish may be contaminated by water contamination)
- Human carriage may persist after untreated infection for months after infection; permanent carrier state is rare.

Key Vignette Clues

Vibrio cholerae

- Patient with noninflammatory diarrhea
- Rice-water stool
- Dehydration from cholera
- Travel to endemic area
- Gram (–) curved rods, polar flagella, oxidase (+)
- Alkaline growth

Transmission

- Fecal-oral spread; sensitive to stomach acid
- Requires high dose (>10^7 organisms), if stomach acid is normal

Pathogenesis

- Motility, mucinase, and toxin coregulated pili (TCP) aid in attachment to the intestinal mucosa.
- **Cholera enterotoxin (choleragen) similar to *E. coli* LT; ADP ribosylates (G_s alpha) activating adenylate cyclase → increased cAMP → efflux of Cl^- and H_2O (persistent activation of adenylate cyclase)**

Disease: cholera

- **Rice water stools**, tremendous fluid loss
- Hypovolemic shock if not treated

Diagnosis: culture stool on TCBS; **oxidase positive**

Treatment: **fluid and electrolyte replacement;** doxycycline or ciprofloxacin to shorten disease and reduce carriage; resistance to tetracycline reported

Prevention: proper sanitation; new vaccine

Other *Vibrio* Species

Table II-2-20. Additional *Vibrio* Species

Species	Reservoir	Transmission	Disease	Symptoms	Treatment
V. parahaemolyticus	Marine life	Consumption of undercooked or raw seafood	Gastroenteritis	Watery diarrhea with cramping and abdominal pain	Self-limiting
V. vulnificus	Brackish water, oysters	Consumption of undercooked or raw seafood	Gastroenteritis	As above	As above
		Swimming in brackish water, shucking oysters	Cellulitis	Rapidly spreading; difficult to treat	Tetracycline; third-generation cephalosporins

FAMILY: ENTEROBACTERIACEAE

Family Features

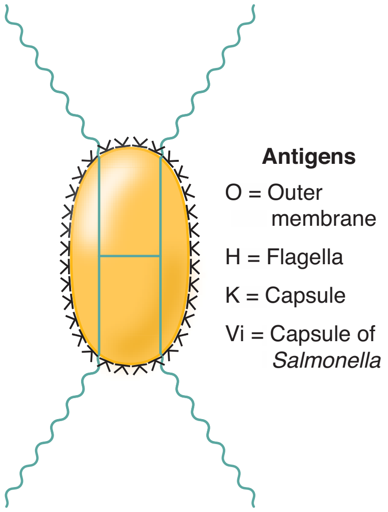
- **Gram-negative rods**
- **Facultative anaerobes**
- Ferment glucose
- **Cytochrome C oxidase negative**
- **Reduce nitrates to nitrites**
- Catalase positive

Family Pathogenesis

- **Endotoxin**; some also produce **exotoxins**
- **Antigens**
  - O: cell envelope or O antigen
  - H: flagellar (motile cells only) antigen
  - K: capsular polysaccharide antigen
  - Vi (virulence): *Salmonella* capsular antigen

Lab Diagnosis

- Blood agar
- Eosin methylene blue or MacConkey agar (differentiate lactose fermentation)
- **Lactose fermenters** (colored colonies)
- **Non–lactose fermenters** (colorless colonies)



Antigens of Enterobacteriaceae

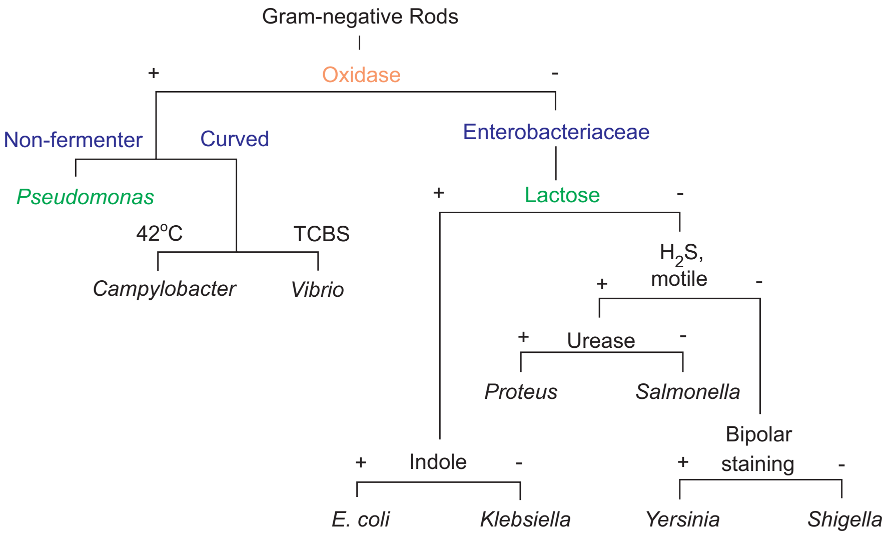


Figure II-2-4. Differentiation of Gram-Negative Rods

GENUS: *ESCHERICHIA*

Genus Features

- **Gram-negative rods**
- **Enterobacteriaceae**
- **Ferments lactose**

Species of Medical Importance—*Escherichia coli*

Escherichia coli

Distinguishing Features

- **Gram-negative rod**
- **Facultative anaerobic, oxidase negative**
- *E. coli* is a **lactose fermenter:** colonies with iridescent green sheen on EMB

Reservoir: **human colon**; may colonize vagina or urethra; contaminated crops where human fecal fertilizer is used; **enterohemorrhagic strains: bovine feces**

Transmission

- Endogenous
- Fecal-oral
- Maternal fecal flora
- Enterohemorrhagic strains: bovine fecal contamination (raw or undercooked beef, milk, apple juice from fallen apples)

Pathogenesis: listed under specific diseases

Note

***E. coli* identification from stool**

- **Isolation of *E. coli* from feces is not by itself significant.**
- **Sorbitol MacConkey screen**
- Most *E. coli* ferment sorbitol.
- Most EHEC do not (colorless)

E. coli from normal floras are more commonly:

- **Immunoassay** looking for specific protein antigens (on or excreted by the bacterium)
- **Serotyping** since certain serotypes are more often pathogenic
- **DNA probes** for specific genes in a culture
- **PCR** on clinical specimen

Mnemonic

Toxins ↑cAMP

c = cholera

A = anthrax

Σ = ETEC LT

P = pertussis

Mnemonic

PITcH

EPEC	P = pediatric
EIEC	I = inflammatory
ETEC	T = traveler
	C = coli
EHEC	H = hamburger

Table II-2-21. Disease Syndromes Caused by *Escherichia coli*

Transmission	Mechanism of Pathogenesis	Clinical Clues	Treatment
UTI Urinary tract infection (most common cause)			
Endogenous fecal flora contaminate and ascend	Motility Adherence to uroepithelium pyelonephritis associated pili, X-adhesins, β hemolytic (many)	Gram (–) bacilli, $\geq 10^5$ CFU/ml	Fluoroquinolone or sulfonamides
Neonatal septicemia/ meningitis (second most common cause)			
Maternal fecal flora contaminate during parturition	Capsule: K1 serotype, endotoxin	Blood, cerebrospinal fluid culture, gram (–) bacilli	Ceftriaxone
Septicemia			
Indwelling IV lines, cytotoxic drugs damage intestinal mucosa, allow escape	Endotoxin	Blood culture, gram (–) bacilli, oxidase (–)	Fluoroquinolones, third-generation cephalosporins
Gastroenteritis			
ETEC (traveler's diarrhea) Fecal/oral	LT: heat-labile toxin stimulates adenylate cyclase by ADP ribosylation of Gs ST: stimulates guanylate cyclase; capsule impedes phagocytosis; colonizing factor adhesins bind to mucosa	Noninflammatory diarrhea, identify enterotoxin by immunoassay, bioassay, DNA probe	Rehydration, TMP/SMX may shorten symptoms
EPEC (second most common infantile diarrhea) Fecal/oral	Adherence to M cells → rearrangement of actin and effacement of brush border microvilli	Noninflammatory diarrhea in babies in developing countries	Beta-lactams
EIEC Fecal/oral	Invades large bowel Inflammatory diarrhea; similar to shigellosis, induces formation of actin jet trails	Inflammatory diarrhea, blood, pus, fever, abdominal pain	
EAEC Fecal/oral	Adherence fimbriae can induce IL-8	Noninflammatory diarrhea in adults and children in United States	Ciprofloxacin if warranted
EHEC (VTEC): O157:H7 most common Bovine feces, petting zoos	Verotoxin: Shigella-like toxins 1 and 2, decreased protein synthesis by interfering with 60S ribosomal subunit	No fever, no PMNs, blood in stool, nonfermenters of sorbitol; may progress to hemorrhagic colitis and HUS; most common in children age <5 years	Antibiotics ↑ risk of hemolytic uremic syndrome

Definition of abbreviations: EIEC, enteroinvasive *E. coli*; EHEC, enterohemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*, *EAEC, enteroaggregative E. coli*

GENUS: *KLEBSIELLA*

Klebsiella pneumoniae

Distinguishing Features

- **Gram-negative rods** with **large** polysaccharide **capsule**
- **Mucoid, lactose-fermenting** colonies on MacConkey agar
- Oxidase negative

Reservoir: human colon and upper respiratory tract

Transmission: endogenous

Pathogenesis : **capsule** (impedes phagocytosis); **endotoxin** (causes fever, inflammation, and shock [septicemia])

Disease(s)

- Pneumonia
  - Community-acquired, most often older men; most commonly **those with chronic lung disease, alcoholism, or diabetes** (but this is not the most common cause of pneumonia in alcoholics; *S. pneumoniae* is.)
  - Endogenous; assumed to reach lungs by inhalation of respiratory droplets from upper respiratory tract
  - Frequent **abscesses** make it hard to treat; fatality rate high
  - **Sputum is generally thick and bloody (currant jelly)** but not foul-smelling as in anaerobic aspiration pneumonia
- Urinary tract infection: **catheter-related (nosocomial)** from fecal contamination of catheters
- Septicemia: in immunocompromised patients, may originate from bowel defect or invasion of IV line

Diagnosis: culture of sputum or clean catch urine sample; lactose fermenter

Treatment: third-generation cephalosporin with or without an aminoglycoside; carbapenem for ESBL-producing strains; KPC strains becoming more predominant

Prevention: good catheter care, limit use

Klebsiella granulomatis

Distinguishing Features: previously called *Calymmatobacterium granulomatis*; facultative-intracellular

Transmission: sexual contact

Key Vignette Clues

Klebsiella pneumoniae

- Elderly patient with typical pneumonia: currant-jelly sputum
- UTI (catheter associated)
- Septicemia: immunocompromised or nosocomial
- Gram (–) bacilli, oxidase (–), encapsulated, lactose fermenters

Note

Comparative Microbiology

Major encapsulated organisms

Some **K**illers **H**ave **P**retty **N**ice **C**apsules:

S*trep pneumoniae*

K*lebsiella pneumoniae*

H*aemophilus influenzae* Type b (a-d)

P*seudomonas aeruginosa*

N*eisseria meningitidis*

C*ryptococcus neoformans* (the yeast)

(Not a complete list, just the big ones)

Disease(s): granuloma inguinale (Donovanosis): small painless nodules that burst and ulcerate; beefy red painless genital ulcers

Diagnosis: Donovan bodies seen on Wright-Giemsa stain; not seen on Gram stain

Treatment: doxycycline

Note

Comparative Microbiology

- Invasive bacteria: PMN in stool: *Shigella, Salmonella, Campylobacter*, EIEC.
- Toxigenic bacteria: ETEC, *V. cholerae, Cl. perfringens,* EHEC.

Key Vignette Clues

Shigella

- Patient with acute bloody diarrhea and fever
- Gram (–) bacilli, which are nonmotile, nonlactose fermenters, do not produce H_2S

GENUS: *SHIGELLA*

Species of Medical Importance

- *Shigella sonnei* (**most common in U.S.**)
- *Shigella flexneri*
- *Shigella dysenteriae* **(most severe disease)**
- *Shigella boydii*

Shigella Species

Distinguishing Features

- **Gram-negative rods,** nonmotile, non-lactose fermenters

Reservoir: human colon only (no animal carriers)

Transmission: fecal-oral spread, person to person

Pathogenesis

- **Endotoxin** triggers inflammation
- No H antigens
- Shigellae **invade M cells** (membrane ruffling and macropinocytosis), get into the cytoplasm, replicate, and then **polymerize actin jet trails to go laterally** without going back out into the extracellular milieu. This produces **very shallow ulcers** and rarely causes invasion of blood vessels.
- **Shiga toxin:**
  - Produced by *S. dysenteriae,* type 1
  - Three activities: **neurotoxic, cytotoxic, enterotoxic**
  - **AB component toxin** is internalized in human cells; **inhibits protein synthesis by clipping 60S ribosomal subunit**

Disease(s): enterocolitis/shigellosis (most severe form is dysentery)

- Few organisms required to start infection (1–10) (extremely acid-resistant)
- 1–4 day incubation
- Organisms invade, producing bloody diarrhea

- **Fever (generally >38.3 C [101.0 F]); lower abdominal cramps; tenesmus; diarrhea first watery, then bloody; invasive but rarely causes septicemia; shallow ulcers**
- Severity depends on age of patient and strain; *S. dysenteriae* type 1 with toxin most severe

Diagnosis: isolation from stool during illness and culture on selective media

Treatment: fluid and electrolyte replacement (mild cases); antibiotics (severe cases); resistance is mediated by plasmid-encoded enzymes; many strains are ampicillin-resistant

Prevention: proper sanitation (sewage, clean drinking water, hand washing)

Aeromonas spp.

Distinguishing Features: oxidase-positive; not Enterobacteriaceae

Transmission: contaminated water or food

Disease(s): inflammatory diarrhea similar to shigellosis (blood and PMNs)

Diagnosis: oxidase-positive, flagellated, glucose-fermenting, gram-negative rods

Treatment: cefixime

GENUS: *YERSINIA*

Yersinia pestis

Distinguishing Features

- Small **gram-negative** rods with **bipolar staining**
- **Facultative intracellular parasite**
- **Coagulase positive**

Reservoir: **zoonosis; U.S. desert southwest: rodents** (e.g., prairie dogs, chipmunks, squirrels); potential **biowarfare agent**

Transmission: wild rodents **flea bite** lead to sylvatic plague; human-to-human transmission by **respiratory droplets**

Pathogenesis

- **Coagulase**-contaminated mouth parts of flea
- **Endotoxin** and exotoxin
- **Envelope antigen (F-1) inhibits phagocytosis.**
- Type III secretion system suppresses cytokine production and resists phagocytic killing

Key Vignette Clues

Yersinia pestis

- Patient with high fever, buboes, conjunctivitis, pneumonia
- Exposure to small rodents, desert Southwest
- Bioterrorism

Disease(s)

- Bubonic plague
  - Flea bites infected animal and then later uninfected human
  - Symptoms: **rapidly increasing fever, regional buboes, conjunctivitis**; if untreated, leads to septicemia and death
- Pneumonic plague
  - Arises from septic pulmonary emboli in bubonic plague or **inhalation of organisms from infected individual**
  - **Highly contagious**
  - Bioterrorism
  - Hemoptysis, chest pain, dyspnea

Diagnosis

- Clinical specimens and **cultures** are hazardous
- **Serodiagnosis** or direct **immunofluorescence**
- **"Safety pin" staining** on blood stain (Wright or Wayson)

Treatment: aminoglycosides

Prevention: animal control and avoidance of sick/dead animals; **killed vaccine** (military)

Yersinia enterocolitica

Distinguishing Features: motile at 25.0 C (77.0 F), nonmotile at 37.0 C (98.6 F); cold growth

Reservoir: zoonotic

Transmission: unpasteurized milk, pork; prominent in northern climates (Michigan, Scandinavia)

Key Vignette Clues

Yersinia enterocolitica

- Patient with inflammatory diarrhea or pseudoappendicitis
- Cold climates
- Unpasteurized milk, pork
- Gram (–) bacilli, non–lactose fermenters, non–H_2S producers

Pathogenesis

- Enterotoxin, endotoxin
- **Multiplies in the cold**

Disease(s)

- Enterocolitis: presentations may vary with age
  - Very young: febrile diarrhea (blood and pus)
  - Older kids/young adults: **pseudoappendicitis** (also caused by *Yersinia pseudotuberculosis*)
  - Adults: enterocolitis with postinfective sequelae like reactive arthritis
- Blood transfusion–associated infections

Diagnosis: stool culture, 25.0 C (77.0 F), cold enrichment

Treatment: supportive care; fluoroquinolone or third-generation cephalosporin for immunocompromised

GENUS: *PROTEUS*

Proteus mirabilis/Proteus vulgaris

Distinguishing Features

- Gram-negative rods, non-lactose fermenting
- **Highly motile;** "swarming" motility on surface of blood agar
- **Urease** produced
- Facultative anaerobe (Enterobacteriaceae), oxidase negative

Reservoir: **human colon and environment (water and soil)**

Transmission: endogenous

Pathogenesis

- **Urease raises urine pH to cause kidney stones** (staghorn renal calculi)
- **Motility** may aid entry into bladder
- Endotoxin causes fever and shock when septicemia occurs

Disease(s): urinary tract infection; septicemia

Diagnosis: culture of blood or urine for lactose-negative organisms with swarming motility

Treatment: fluoroquinolone, TMP-SMX, or third-generation cephalosporin for uncomplicated UTI; remove stones if present

Prevention: promptly remove urinary tract catheter

Key Vignette Clues

Proteus mirabilis/Proteus vulgaris

- Patient with UTI or septicemia
- Swarming motility
- Staghorn renal calculi (struvite stones)
- Gram (–), non–lactose fermenting, urease (+)

Note

Weil-Felix test: antigens of OX strains of *Proteus vulgaris* cross-react with rickettsial organisms.

GENUS: *SALMONELLA*

Species of Medical Importance

There are >2,400 serotypes of *Salmonella*.

- *S. typhi*
- *S. enteritidis*
- *S. typhimurium*
- *S. choleraesuis*
- *S. paratyphi*
- *S. dublin*

Key Vignette Clues

Salmonella typhi

- Patient with fever, abdominal pain
- Travel to endemic area
- Gram (–), encapsulated, nonlactose fermenter, produces H_2S gas
- Widal test

Salmonella enterica typhi

Distinguishing Features

- **Gram-negative rods,** highly motile with the **Vi capsule**
- Facultative anaerobe, **non–lactose fermenting**
- **Produces H_2S**
- Species identification with biochemical reactions
- **Sensitive to acid**

Reservoir: **humans only; no animal reservoirs**

Transmission: **fecal-oral** route from **human carriers (gall bladder)**; **decreased stomach acid or** impairment of mononuclear cells as in **sickle cell disease predisposes** to *Salmonella* infection

Pathogenesis and Disease: typhoid fever (enteric fever), ***S. typhi*** (milder form: paratyphoid fever; *S. paratyphi*)

- **Infection begins in ileocecal region**; **constipation** common
- Host cell membranes "ruffle" from *Salmonella* contact.
- *Salmonella* reach basolateral side of M cells, then **mesenteric lymph nodes and blood** (transient 19 septicemia)
- At 1 week: patients have 80% **positive blood cultures**; 25% have **rose spots** (trunk/abdomen), signs of **septicemia** (mainly fever)
- *S. typhi* survives intracellularly and replicates in macrophages; **resistant to macrophage killing** because of **decreased fusion of lysosomes with phagosomes** and **defensins** (proteins) allow it to withstand oxygen-dependent and oxygen-independent killing
- **By week 3: 85% of stool cultures** are positive
- Symptoms: **fever,** headache, abdominal pain, constipation more common than diarrhea
- Complications if untreated: **necrosis of Peyer patches** with perforation (local endotoxin triggered damage), thrombophlebitis, cholecystitis, pneumonia, abscess formation, etc.

Diagnosis: organisms can be isolated from blood, bone marrow, urine, and tissue biopsy from the rose spots if present; antibodies to O, Vi, and H antigens in patient's serum can be detected by agglutination (**Widal test**)

Treatment: fluoroquinolones or third-generation cephalosporins

Prevention: sanitation; 3 vaccines (attenuated oral vaccine of *S. typhi* strain 21 (Ty21a), parenteral heat-killed *S. typhi* (no longer used in U.S.), and parenteral ViCPS polysaccharide capsular vaccine)

Salmonella Subspecies other than *typhi* (*S. enteritidis*, *S. typhimurium*)

Distinguishing Features

- Facultative gram-negative rods, non–lactose-fermenting on EMB, MacConkey medium
- Produces H_2S, **motile** (unlike *Shigella*)
- Speciated with biochemical reactions and serotyped with O, H, and Vi antigens

Reservoir: enteric tracts of humans and domestic animals, e.g., **chickens** and turtles

Transmission: raw chicken and eggs in kitchen; food-borne outbreaks (peanut butter, produce, eggs); reptile pets (snakes, turtles)

Pathogenesis

- Sensitive to stomach acid (infectious dose 10^5 organisms)
- Lowered stomach acidity (antacids or gastrectomy) increases risk
- Endotoxin in cell wall; no exotoxin
- **Invades** mucosa in ileocecal region, invasive to lamina propria → **inflammation** → **increased PG** → **increased cAMP** → loose diarrhea; shallow ulceration
- Spread to septicemia not common with *S. enterica* subsp. *enteritidis* (the most common) but may occur with others

Disease(s)

- Enterocolitis/gastroenteritis (second most common bacterial cause after *Campylobacter*): **6–48 hour incubation; nausea; vomiting; only occasionally bloody, loose stools; fever; abdominal pain; myalgia; headache**
- Septicemia (*S. enterica* subsp. *choleraesuis, S. enterica* subsp. *paratyphi,* and *S. enterica* subsp. *dublin)*: usually in very young or elderly when it occurs; endocarditis or arthritis complicates 10% of cases
- Osteomyelitis: sickle cell disease predisposes to osteomyelitis; *Salmonella* is **most common causal agent of osteomyelitis in sickle cell disease** (not trait) **patients (>80%)**

Diagnosis: culture on Hektoen agar, H_2S production

Treatment: antibiotics are contraindicated for self-limiting gastroenteritis; ampicillin, third-generation cephalosporin, fluoroquinolone, or TMP-SMX for invasive disease

Prevention: properly cook foods and wash hands, particularly food handlers

Key Vignette Clues

Salmonella enterica Subspecies Other Than *typhi*

- Enterocolitis—inflammatory, follows ingestion of poultry products or handling pet reptiles
- Septicemia—very young or elderly
- Osteomyelitis—sickle cell disease
- Gram (–) bacillus, motile, non–lactose fermenter, produces H_2S

GENUS: *GARDNERELLA*

Gardnerella vaginalis

Distinguishing Features

- Gram-variable rod; has Gram-positive cell envelope
- Facultative anaerobe
- Catalase-negative and oxidase-negative

Reservoir: human vagina

Transmission: endogenous (normal flora gets disturbed, increased pH)

Pathogenesis

- Polymicrobial infections
- Works synergistically with other normal flora organisms including *Lactobacillus, Mobiluncus, Bacteroides, Peptostreptococcus*
- Thought to flourish when the vaginal pH increases, reduction of vaginal *Lactobacillus*
- Follows menses or antibiotic therapy

Disease: bacterial vaginosis (**vaginal odor, increased discharge** (thin, gray, adherent fluid)

Diagnosis: pH >4.5, **clue cells** (epithelial cells covered with bacteria) on vaginal saline smear; for Whiff test, add KOH to sample and assess for "fishy" amine odor

Treatment: metronidazole or clindamycin

Key Vignette Clues

Gardnerella

- Female patient with thin vaginal discharge
- Post antibiotic or menses
- Clue cells
- Whiff test

GENUS: *BACTEROIDES*

Bacteroides fragilis

Distinguishing Features: anaerobic gram-negative rods; modified LPS with reduced activity

Reservoir: human **colon;** the genus ***Bacteroides*** is predominant anaerobe

Transmission: **endogenous** from bowel defects (e.g., cytotoxic drug use, cancer), surgery, or trauma

Pathogenesis: modified LPS (missing heptose and 2-keto-3 deoxyoctonate) has reduced endotoxin activity; capsule is antiphagocytic

Diseases: **septicemia, peritonitis (often mixed infections), and abdominal abscess**

Diagnosis: anaerobes are identified by biochemical tests and gas chromatography

Key Vignette Clues

Bacteroides fragilis

- Patient with abdominal trauma, emergency abdominal surgery
- Septicemia, peritonitis, abscess
- Gram (–) bacilli, anaerobic

Treatment

- Metronidazole, clindamycin, or cefoxitin; **abscesses should be surgically drained**
- **Antibiotic resistance** common (penicillin G, some cephalosporins, and aminoglycosides); 7–10% of all strains now clindamycin-resistant

Prevention: prophylactic antibiotics for GI or biliary tract surgery

Porphyromonas, Prevotella, Fusobacterium spp.

Distinguishing Features: Gram-negative rods, anaerobic, normal oral flora

Transmission: endogenous

Pathogenesis: *Porphyromonas* has gingipains: act as proteases, adhesins, degrades IgG antibodies and inflammatory cytokines

Disease: periodontal disease

Diagnosis: anaerobic, gram-negative rods isolated from abscess

Treatment: metronidazole

SPIROCHETES

GENUS: *TREPONEMA*

Treponema pallidum

Distinguishing Features

- **Thin spirochete,** not reliably seen on Gram stain (basically a gram-negative cell envelope)
- Outer membrane has endotoxin-like lipids
- Axial filaments = endoflagella = periplasmic flagella
- Cannot culture in clinical lab; serodiagnosis
- Is an **obligate pathogen (but not intracellular)**

Reservoir: human genital tract

Transmission: transmitted **sexually** or **across the placenta**

Pathogenesis: disease characterized by **endarteritis resulting in lesions**; strong tendency to chronicity

Key Vignette Clues

Treponema pallidum

- Sexually active patient or neonate of IV drug-using female
- **Primary**: nontender, indurated genital chancre
- **Secondary**: maculopapular, copper-colored rash, condylomata lata
- **Tertiary**: gummas in CNS and cardiovascular system
- Spirillar, gram (–) bacteria visualized by dark-field or fluorescent antibody
- Specific and nonspecific serologic tests

Table II-2-22. Stages of Syphilis

Stage	Clinical	Diagnosis
Primary (10 d to 3 mo post-exposure)	Nontender chancre; clean, indurated edge; contagious; heals spontaneously 3–6 weeks	**Fluorescent microscopy of lesion** 50% of patients will be negative by nonspecific serology
Secondary (1 to 3 mo later)	Maculopapular (copper-colored) rash, diffuse, includes palms and soles, patchy alopecia Condylomata lata: flat, wartlike perianal and mucous membrane lesions; highly infectious	**Serology nonspecific and specific;** both positive
Latent	None	Positive serology
Tertiary (30% of untreated, years later)	Gummas (syphilitic granulomas), aortitis, CNS inflammation (tabes dorsalis)	**Serology: specific tests** Nonspecific may be negative
Congenital (babies of IV drug–using)	Stillbirth, keratitis, 8th nerve damage, notched teeth; most born asymptomatic or with rhinitis → widespread desquamating maculopapular rash	Serology: should revert to negative within 3 mo of birth if uninfected

Diagnosis

- **Visualize organisms by immunofluorescence or microscopy** (dark field microscopy was standard but no longer used)
- **Serology** important: 2 types of antibody:
  - **Nontreponemal antibody** (= **reagin) screening tests**
    - **Ab binds to cardiolipin**: antigen found in mammalian mitochondrial membranes and treponemes; cheap source of antigen is cow heart, used in screening tests (VDRL, RPR, ART); very sensitive in primary (except early) and secondary syphilis; titer may decline in tertiary and with treatment; not specific so confirm with FTA-ABS
    - **Examples:** venereal disease research lab (VDRL), rapid plasma reagin (RPR), automated reagin test (ART), recombinant antigen test (ICE)
  - **Specific tests for treponemal antibody (more expensive)**
    - **Earliest** antibodies; **bind to spirochetes**: these tests are more specific and positive earlier; usually remain positive for life, but positive in those with other treponemal diseases (bejel) and may be positive in Lyme disease; fluorescent treponemal antibody-absorption **(FTA-ABS; most widely used test**); *Treponema pallidum* microhemagglutination (MHA-TP)

Treatment

- **Benzathine penicillin** (long-acting form) for primary and secondary syphilis (no resistance to penicillin); penicillin G for congenital and late syphilis
- Jarisch-Herxheimer reaction: starts during first 24 hours of antibiotic treatment; increased temperature and decreased BP; rigors, leukopenia; may occur during treatment of **any spirochete disease**

Prevention: benzathine penicillin given to contacts; no vaccine available

GENUS: *BORRELIA*

Genus Features

- **Larger spirochetes**
- **Gram negative**
- **Microaerophilic**
- **Difficult to culture**

Borrelia burgdorferi

Reservoir: white-footed mice (nymphs) and **white-tailed deer (adult ticks)**

Transmission: *Ixodes* (deer) ticks and nymphs; worldwide but in 3 main areas of U.S.:

- ***Ixodes scapularis*** *(I. dammini)* in Northeast (e.g., Connecticut), Midwest (e.g., Wisconsin, Minnesota)
- ***Ixodes pacificus*** on West Coast (e.g., California)
- Late spring/early summer incidence

Pathogenesis: *B. burgdorferi* **invades skin and spreads via bloodstream** to involve primarily the heart, joints, and CNS; arthritis is caused by immune complexes

Disease: Lyme disease (#1 vector-borne disease in U.S.)

Key Vignette Clues

Borrelia burgdorferi

- Patient with influenza-like symptoms and erythema migrans
- Spring/summer seasons
- Northeast, Midwest, West Coast
- Later: neurologic, cardiac, arthritis/arthralgias

Stage 1: early localized (3 days to 1 month)	Target rash Flu-like symptoms
Stage 2: early disseminated (days to weeks) (**organism spreads hematogenously**)	Swollen lymph nodes Secondary annular skin lesions Bell palsy, headache, meningitis, extreme fatigue, conjunctivitis Palpitations, arrhythmias, myocarditis, pericarditis
Stage 3: late persistent (months to years)	Arthritis (mostly knees), immune complex-mediated

Diagnosis: **serodiagnosis** by ELISA (negative early); Western blot for confirmation

Treatment: doxycycline, amoxicillin, or azithromycin/clarithromycin for primary; ceftriaxone for secondary; doxycycline or ceftriaxone for arthritis

Prevention: DEET; avoid tick bites; vaccine (OspA flagellar antigen) not used in U.S.

Borrelia recurrentis and B. hermsii

Distinguishing Features: spirochetes, cause relapsing fever

Transmission: human body louse for *B. recurrentis*; soft ticks from mice for *B. hermsii* (and 13 other species of Borrelia)

Pathogenesis: antigenic variation leads to return of fever/chills

Disease(s): relapsing fever (tick-borne relapsing fever in U.S. is caused mainly by *B. hermsii*); associated with camping in rural areas of Colorado

Diagnosis: spirochetes seen on dark-field microscopy of blood smear when patient is febrile

Treatment: doxycycline; Jarisch-Herxheimer reaction possible

GENUS: *LEPTOSPIRA*

Leptospira interrogans

Distinguishing Features: **spirochetes with tight terminal hooks or coils** (seen on **dark-field microscopy** but not light; can be cultured in vitro; aerobic); generally diagnosed by serology

Reservoir: wild and domestic animals (zoonosis)

Transmission

- **Contact with animal urine in water; organism penetrates mucous membranes or enters small breaks in epidermis**
- In U.S., **via dog, livestock,** and **rat urine** through contaminated **recreational waters (jet skiers) or occupational exposure (sewer workers)**
- **Hawaii highest incidence state**

Pathogenesis: no toxins or virulence factors known

Disease: leptospirosis (swineherd's disease, swamp or mud fever); **influenza-like disease ± GI tract symptoms (Weil disease)**; if not treated, can progress to hepatitis and renal failure

Diagnosis: serodiagnosis (agglutination test); culture (blood, CSF, urine) available in few labs; dark-field microscopy insensitive

Treatment: penicillin G or doxycycline

Prevention: doxycycline for short-term exposure; vaccination of domestic livestock and pets; rat control

Key Vignette Clues

Leptospira interrogans

- Patients with influenza-like symptoms ± GI symptoms
- Occupational or recreational exposure to water aerosols
- Hawaii
- Spirochetes with terminal hook

UNUSUAL BACTERIA

Table II-2-23. Comparison of *Chlamydiaceae*, *Rickettsiaceae*, and *Mycoplasmataceae* with Typical Bacteria

	Typical Bacteria (*S. aureus*)	Chlamydiaceae	Rickettsiaceae	Mycoplasmataceae
Obligate intracellular parasite?	Mostly no	Yes	Yes	No
Make ATP?	Normal ATP	No ATP	Limited ATP	Normal ATP
Peptidoglycan layer in cell envelope?	Normal peptidoglycan	Modified* peptidoglycan	Normal peptidoglycan	No peptidoglycan

*Chlamydial peptidoglycan lacks muramic acid and is considered by some as modified, by others as absent.

FAMILY: CHLAMYDIACEAE

Family Features

- **Obligate intracellular bacteria**
- **Elementary body/reticulate body**
- **Not seen on Gram stain**
- **Cannot make ATP**
- **Cell wall lacks muramic acid**

Genera of Medical Importance

- *Chlamydia trachomatis*
- *Chlamydophila pneumoniae*
- *Chlamydophila psittaci*

Chlamydia trachomatis

Distinguishing Features

- Obligate intracellular bacterium; cannot make ATP
- Found **in cells as metabolically active, replicating reticulate bodies**
- **Infective form:** inactive, extracellular **elementary body**
- Not seen on Gram stain; peptidoglycan layer lacks muramic acid

Key Vignette Clues

Chlamydia trachomatis

- Sexually active patient or neonate
- **Adult**: urethritis, cervicitis, PID, inclusion
- conjunctivitis
- **Neonate**: inclusion conjunctivitis/ pneumonia
- Immigrant from Africa/Asia, genital lymphadenopathy
- Cytoplasmic inclusion bodies in scrapings

Reservoir: **human** genital tract and eyes

Transmission: **sexual contact** and **at birth;** trachoma is transmitted by **hand-to-eye contact** and flies.

Pathogenesis: **infection of nonciliated columnar or cuboidal epithelial cells of mucosal surfaces** leads to **granulomatous response and damage**

Diseases

- STDs in U.S.
  - **Serotypes D-K** (most common **bacterial** STD in U.S., though overall herpes and HPV are more common in prevalence)
  - **Nongonococcal urethritis, cervicitis, PID,** and major portion of infertility (no resistance to reinfection)
  - **Inclusion conjunctivitis** in adults (with NGU and reactive arthritis)
  - **Inclusion conjunctivitis** and/or **pneumonia in neonates/infants (staccato cough)** with eosinophilic infiltrate
- Lymphogranuloma venereum
  - **Serotypes L1, 2, 3** (prevalent in Africa, Asia, South America); painless ulcer at site of contact; swollen lymph nodes (buboes) around inguinal ligament (groove sign); tertiary includes ulcers, fistulas, genital elephantiasis
- Trachoma
  - Leading cause of preventable infectious blindness: **serotypes A, B, Ba, and C**
  - **Follicular conjunctivitis** leading to **conjunctival scarring**, and **inturned eyelashes** leading to **corneal scarring** and **blindness**

Diagnosis

- NAAT; DNA probes in U.S. (rRNA) and PCR
- **Cytoplasmic inclusions seen on Giemsa-, iodine-, or fluorescent-antibody-stained smear or scrapings**
- **Cannot be cultured on inert media**
- Is cultured in **tissue cultures or embryonated eggs**
- Serodiagnosis: DFA, ELISA

Treatment: azithromycin or doxycycline

Prevention: erythromycin for infected mothers to prevent neonatal disease; systemic erythromycin for neonatal conjunctivitis to prevent pneumonia

GENUS: *CHLAMYDOPHILA*

Table II-2-24. Diseases Caused by *Chlamydophila* Species

Organism	*C. pneumoniae*	*C. psittaci*
Distinguishing characteristics	Potential association with atherosclerosis	No glycogen in inclusion bodies
Reservoir	Human respiratory tract	Birds, **parrots,** turkeys (major U.S. reservoir)
Transmission	Respiratory droplets	Dust of dried bird secretions and feces
Pathogenesis	Intracellular growth; infects smooth muscle, endothelial cells, or coronary artery and macrophages	Intracellular growth
Disease	Atypical "walking" pneumonia; single lobe; bronchitis; scant sputum, prominent dry cough and hoarseness; sinusitis	Psittacosis (ornithosis); atypical pneumonia with hepatitis, possible CNS and GI symptoms
Diagnosis	Serology (complement fixation or microimmunofluorescence) Cold-agglutinin negative	Serology, complement fixation Cold-agglutinin negative
Treatment	Macrolides and tetracycline	Doxycycline
Prevention	None	Avoid birds

Key Vignette Clues

Chlamydophila

- *C. pneumoniae:* atypical pneumonia: sputum with intracytoplasmic inclusions
- *C. psittaci:* atypical pneumonia: exposure to parrots

GENUS: *RICKETTSIA*

Table II-2-25. Infections Caused by Rickettsiae and Close Relatives

Group Disease	Bacterium	Arthropod Vector	Reservoir Host
Rocky Mountain Spotted Fever	*R. rickettsii*	Ticks	Ticks, dogs, rodents
Epidemic Typhus	*R. prowazekii*	Human louse	Humans
Endemic Typhus	*R. typhi*	Fleas	Rodents
Scrub Typhus	*Orientia tsutsugamushi*	Mites	Rodents
Ehrlichiosis	*E. chaffeensis* *A. phagocytophilum*	Tick	Small mammals

Genus Features

- **Aerobic, gram-negative bacilli (too small to stain well with Gram stain)**
- **Obligate intracellular bacteria (do not make sufficient ATP for independent life)**

Species of Medical Importance

- *Rickettsia rickettsii*
- *Rickettsia prowazekii*
- *Rickettsia typhi*
- *Orientia tsutsugamushi* (formerly *R. tsutsugamushi*)
- *Ehrlichia spp.*
- *Coxiella burnetii*

Key Vignette Clues

Rickettsia rickettsii

- Patient with influenza-like symptoms and petechial rash that begins on ankles and wrists and moves to trunk
- East Coast mountainous areas
- Spring/summer seasons
- Outdoor exposure
- Weil-Felix (+)

Rickettsia rickettsii

Reservoir: small wild rodents and larger wild and domestic animals (dogs)

Transmission: **hard ticks: *Dermacentor* (also reservoir hosts** because of trans-ovarian transmission)

Pathogenesis: **invade endothelial cells lining capillaries, causing vasculitis in many organs including brain, liver, skin, lungs, kidney, and GI tract**

Disease: Rocky Mountain spotted fever (RMSF)

- Prevalent on **East Coast (OK, TN, NC, SC)**; 2–12 day incubation
- **Headache, fever (38.8 C [102.0 F])**, malaise, myalgias, toxicity, vomiting, and confusion
- **Rash** (maculopapular → **petechial) starts** (by day 6 of illness) **on ankles and wrists** and then spreads to the trunk, **palms, soles, and face (centripetal rash)**
- **Ankle and wrist swelling** also occur
- Diagnosis may be confused by GI symptoms, periorbital swelling, stiff neck, conjunctivitis, and arthralgias

Diagnosis

- Clinical symptoms (above) and tick bite
- **Start treatment** without laboratory confirmation
- **Serological IFA test most widely used; fourfold increase in titer is diagnostic**
- Weil-Felix test (cross-reaction of *Rickettsia* antigens with OX strains of *Proteus vulgaris*) is no longer used (but may still be asked!)

Treatment: doxycycline, even in children age <8 years

Prevention: tick protection and prompt removal; doxycycline for exposed persons

GENUS: *EHRLICHIA*

Genus Features

- **Gram-negative bacilli**
- **Obligate intracellular bacteria of mononuclear or granulocytic phagocytes**

Species of Medical Importance

- *Ehrlichia chaffeensis*
- *Anaplasma phagocytophilum*

Ehrlichia chaffeensis/Anaplasma phagocytophilum

Table II-2-26. Diseases Caused by *Ehrlichia* Species

Organism	Reservoir	Transmission	Pathogenesis	Disease	Diagnosis	Treatment
E. chaffeensis	Ticks and deer	Lone star tick (*Amblyomma*)	Infects monocytes and macrophages	Ehrlichiosis (monocytic) similar to RMSF without rash; leukopenia, low platelets, morulae	IFA PCR Blood film **Morulae**	Doxycycline
A. phagocytophilum	Ticks, deer, mice	*Ixodes* ticks Coinfection with *Borrelia*	Infects neutrophils	Ehrlichiosis (granulocytic) similar to RMSF without rash; leukopenia, low platelets, morulae	IFA PCR Blood film **Morulae**	Doxycycline

Disease

- Similar to Rocky Mountain spotted fever but generally without rash
- Leukopenia
- Thrombocytopenia
- Morulae: mulberry-like structures inside infected cells

Diagnosis: Giemsa-stained blood film (**morulae**); serology; DNA probe

Treatment: doxycycline (begin before lab confirmation)

Prevention: no vaccine, avoid ticks

Key Vignette Clues

Ehrlichia chaffeensis/Anaplasma phagocytophilum

- Patient with influenza-like symptoms, no rash, leukopenia, thrombocytopenia
- Same geographic range as Lyme disease
- Spring/summer seasons
- Exposure to outdoors
- Morulae inside monocytes or granulocytes

Coxiella burnetii

Distinguishing Features: obligate intracellular, spore-like characteristics

Transmission: inhalation from dried placental material; zoonosis (sheep and goats); possible bioterrorism agent

Pathogenesis: obligate intracellular, live inside phagolysosomes

Disease(s): Q fever: atypical pneumonia, hepatitis, or endocarditis

Diagnosis: serologic detection of Phase II LPS antigen (for acute disease) and Phase I and Phase II LPS antigens (for chronic disease)

Treatment: doxycycline

FAMILY: MYCOPLASMATACEAE

Family Features

- **Smallest free-living (extracellular) bacteria**
- **Missing peptidoglycan (no cell wall)**
- **Sterols in membrane**
- **Require cholesterol for in vitro culture**
- **"Fried-egg" colonies on *Mycoplasma* or Eaton's media**

Genera of Medical Importance

- *Mycoplasma pneumoniae*
- *Ureaplasma urealyticum*

Mycoplasma pneumoniae

Distinguishing Features

- Extracellular, tiny, flexible
- No cell wall; not seen on Gram-stained smear
- Membrane with cholesterol but **does not synthesize cholesterol**
- Requires cholesterol for in vitro culture

Reservoir: human respiratory tract

Transmission: respiratory droplets; close contact: families, military recruits, medical school classes, college dorms

Key Vignette Clues

Mycoplasma pneumoniae

- Young adult with atypical pneumonia
- Mulberry-shaped colonies on media containing sterols
- Positive cold agglutinin test

Pathogenesis

- Surface parasite: not invasive
- **Attaches to respiratory epithelium via P1 protein**
- **Inhibits ciliary action**
- **Produces hydrogen peroxide, superoxide radicals, and cytolytic enzymes,** which damage the respiratory epithelium, leading to necrosis and a bad, hacking cough (walking pneumonia)
- *M. pneumoniae* functions as superantigen, elicits production of IL-1, IL-6, and TNF-α

Disease: walking pneumonia

- **Pharyngitis**
- May develop into atypical pneumonia with persistent hack (**little sputum produced)**
- **Most common atypical pneumonia (along with viruses) in young adults**

Diagnosis

- Primarily clinical diagnosis; PCR/nucleic acid probes
- **ELISA and immunofluorescence sensitive and specific**
- **Fried-egg-shaped colonies on sterol-containing media, 10 days**
- **Positive cold agglutinins** (autoantibody to RBCs) test is nonspecific and is positive in only 65% of cases

Treatment: erythromycin, azithromycin, clarithromycin; **no cephalosporin or penicillin**

Prevention: none

Ureaplasma urealyticum

Distinguishing Features: member of family Mycoplasmataceae

Pathogenesis: urease positive

Diseases: urethritis, prostatitis, renal calculi

Diagnosis: non-Gram-staining, urease(+)

Treatment: erythromycin or tetracycline

Key Vignette Clues

Ureaplasma urealyticum

- Adult patient with urethritis, prostatitis, renal calculi
- Alkaline urine
- Non–Gram-staining, urease (+)

Bacterial Genetics 3

Learning Objectives

- ❑ Explain how DNA is rearranged within a bacterium
- ❑ Answer questions about bacterial gene transfer, mechanisms of bacterial DNA exchange and conjugal crosses
- ❑ Demonstrate understanding of drug resistance
- ❑ Explain information related to antibiotic susceptibility testing

BACTERIAL GENETIC MATERIAL

Three types of DNA may be found in a bacterial cell: **bacterial chromosomal** DNA, **plasmid** DNA, or **bacteriophage** DNA that is integrated into the bacterial genome.

Bacterial Chromosome (Genome)

Most bacteria have only **1 chromosome but there are often multiple copies** of it in the cell. Most bacterial chromosomes are a **large, covalently closed, circular DNA molecule**.

- The chromosome is **organized into loops** around a proteinaceous center. A single-stranded topoisomerase (1 nick) will relax only the nicked loop, allowing DNA synthesis or transcription.
- Most have **around 2,000 genes**.
- All **essential genes** are on the bacterial chromosome.

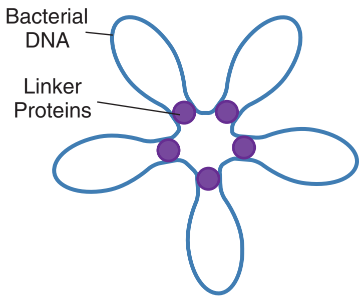


Figure II-3-1. Bacterial Chromosome

Plasmids

Plasmids are **extrachromosomal genetic elements** found in bacteria (and eukaryotes).

- Generally covalently closed, **circular DNA**
- **Small** (1.5–400 kB)
- Can **replicate autonomously** in bacterial cells

One subclass of plasmids, **episomes**, may be integrated into the bacterial DNA. Episomes have insertion sequences matching those on the bacterial chromosome.

Plasmids carry the genetic material for a variety of genes, e.g., the **fertility genes** directing conjugation (*tra* operon), many genes for **antibiotic resistance**, and most **bacterial exotoxins**. They contain genes that are **nonessential** for bacterial life.

Bacteriophage (= Phage = Bacterial Virus) Genome

Stable pieces of bacteriophage DNA may be present in the bacterial cell. These are **generally repressed temperate phage** (called **prophage)** inserted into the bacterial chromosome.

- Besides the repressor protein, prophage DNA may also direct synthesis of other proteins. Most notable are gene products that make bacteria more pathogenic.
- This enhanced virulence is called **lysogenic conversion.**

GENE TRANSFER

Bacterial reproduction is by the **asexual process of binary fission**. With the exception of a de novo mutation, the resultant daughter cells are genetically identical to the parent cell. This lends itself to the question, "How then have bacteria undergone genetic variation resulting in the different virulence factors and antibiotic resistances?"

Three mechanisms have been observed to transfer novel genetic material into bacteria: **transformation**, **conjugation**, and **transduction**.

Upon reception of the new genes, the genetic material must be stabilized either by reformation of a plasmid or by recombination. Linear DNA is always stabilized by homologous recombination.

Occasionally, a plasmid will be an episome and integrate into the bacterial chromosome by the process of site-specific recombination.

Transformation

Transformation is the **uptake of naked (free) DNA from the environment by competent cells.**

- Cells become competent (**able to bind short pieces of DNA to the envelope and import them into the cell**) under certain environmental conditions (which you do not need to know).
- Some bacteria are capable of **natural transformation** (they are naturally competent): *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus* species, and *Neisseria* species.
- DNA (released from dead cells) is taken up.
- Newly introduced DNA is generally linear, homologous DNA; a similar type of cell but perhaps one that is genetically diverse.
- The linear DNA is then stabilized by homologous recombination.

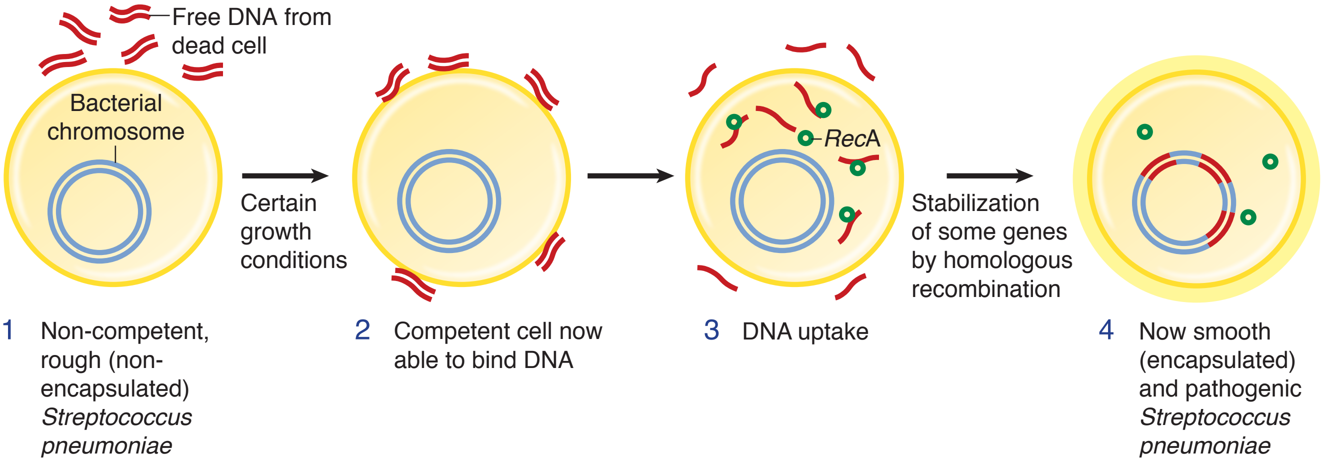
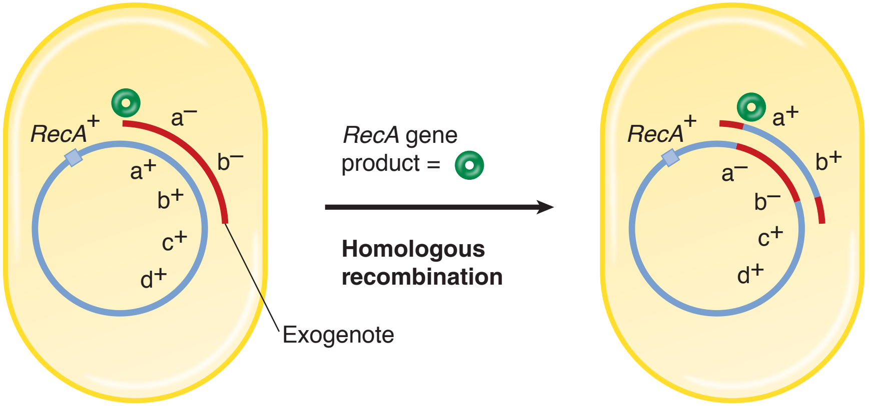


Figure II-3-2. Transformation of a Nonencapsulated *Streptococcus Pneumoniae*

Homologous Recombination

Homologous recombination is a gene exchange process that may **stabilize genes** introduced into a cell by transformation, conjugation, or transduction. Imported bacterial genes (transferred into a cell by transformation, conjugation, or transduction) are on short linear pieces of DNA called **exogenotes**. Linear DNA is not stable in cells because it is broken down by exonucleases.

- Produces an **"exchange"** of DNA between the linear exogenote of DNA and a homologous region on the stable (circular) bacterial chromosome
- Requires several genes worth of **homology or near homology** between the DNA strands, plus a series of recombination **enzymes**/factors coded for by the recombination genes ***recA***, *recB*, *recC*, and *recD* (with *recA* an absolute requirement)



Genes ending up on the linear piece of DNA are lost. Those on the circular molecule become part of the cell's permanent genetic make up.

Figure II-3-3. Homologous Recombination

Conjugation

Conjugation is **gene transfer from one bacterial cell to another** involving direct cell-to-cell contact. In a conjugal mating, a donor cell will transfer a single strand of DNA to a recipient cell. Donor cells contain fertility factors that encode for gene products involved in conjugation.

There are 2 types of donor cell:

- **F+ cells**: fertility factors in a plasmid
- **Hfr cells**: fertility factors in an episome

All recipient cells are devoid of fertility factor.

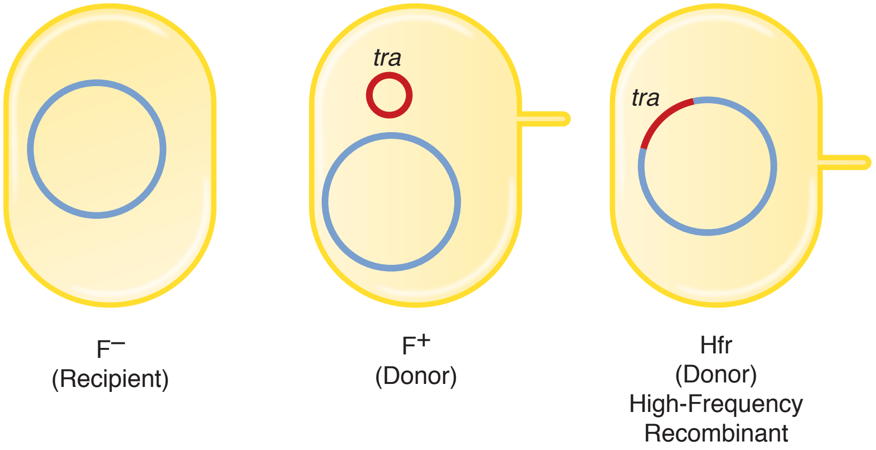


Figure II-3-4. Mating Types of Bacteria

Donor (male) cells: F^+ and Hfr cells

All donor cells have fertility plasmids known as **F factors**. F factors control conjugation through a series of important "fertility" genes called the transfer or ***tra* region**. The *tra* region code for the following:

- Sex pili (play a role in establishing cell-to-cell contact)
- Genes whose products **stabilize mating pairs**
- Genes that **direct conjugal DNA transfer**, and other genes

Donor cells have a region called ***oriT* (origin of transfer)**, where a single strand break in the DNA will be made and then *oriT* begins the transfer of one strand of the double helix. Many have **insertion sequences**, where the plasmid can be inserted into the bacterial chromosome, combining to make one larger molecule of DNA.

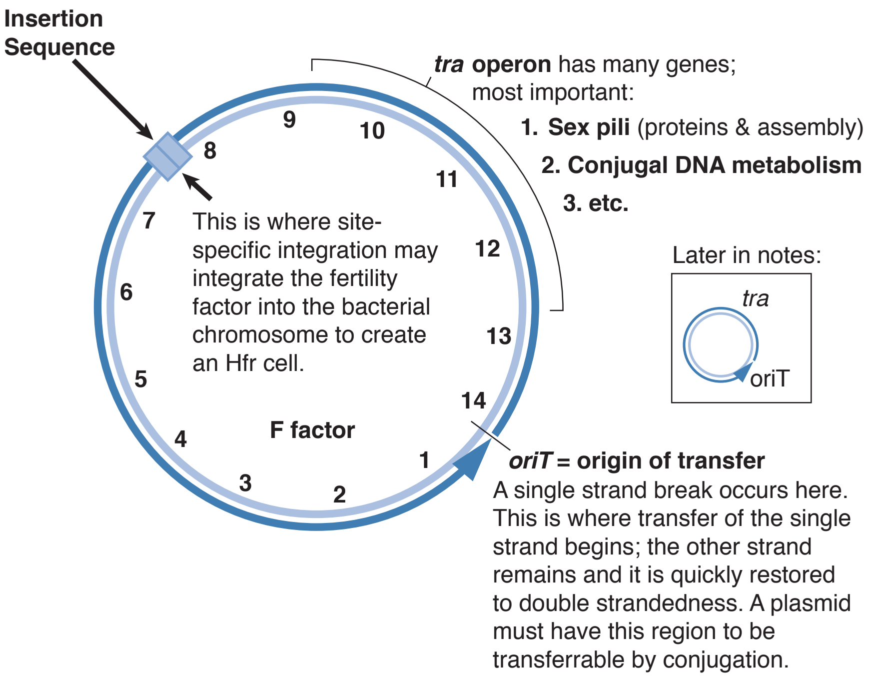


Figure II-3-5. Genetic Map of a Fertility (F)

- Donor cells in which the **fertility plasmid** is in its **free state** are called **F^+ cells**.
- Donor cells in which the **fertility factor has inserted** itself into the bacterial chromosome are called **Hfr cells**. An integrated plasmid is called an episome.

Recipient (female) cells: F^- cells

Recipient cells **lack fertility factors**. In every cross, one cell must be an F^- cell.

Site-Specific Recombination

Site-specific recombination is the integration of one DNA molecule into another DNA molecule with which it has **no homology** except for a small site on each DNA (called an **attachment, integration, or insertion site**).

- Requires **restriction endonucleases** and restriction endonuclease sites on each DNA
- Because this process **integrates** rather than exchanges pieces of DNA, the end result is a molecule the **sum of the 2 original molecules**.

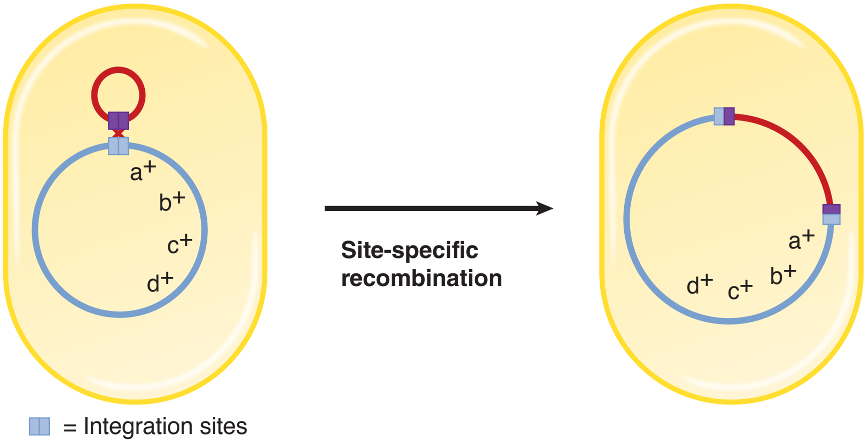


Figure II-3-6. Site-Specific Recombination

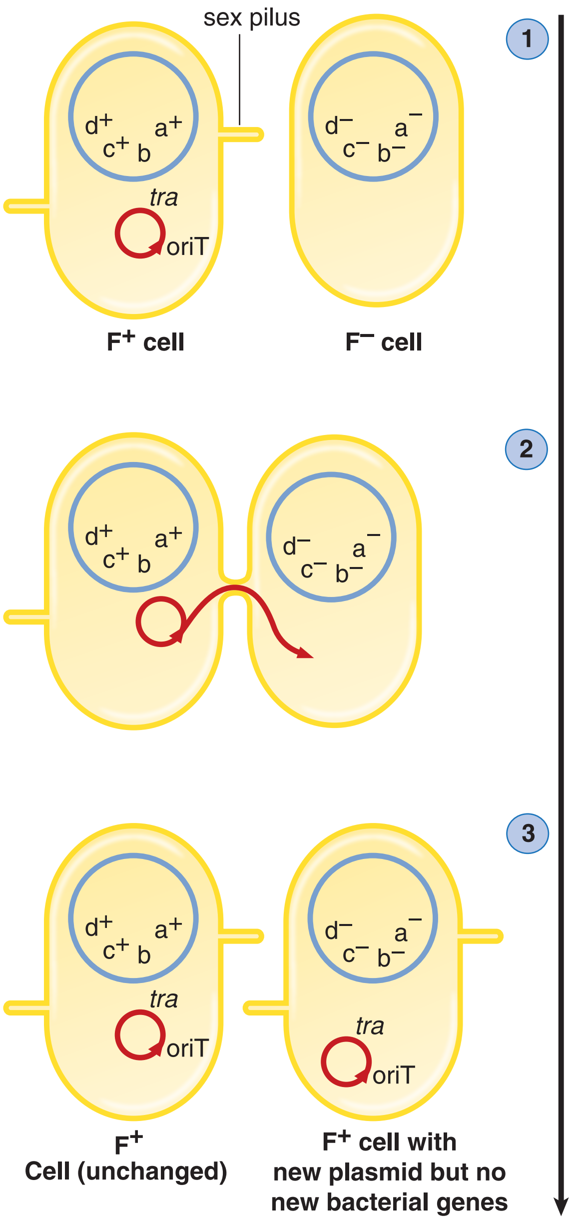
Note

Transposition is the name of site-specific integration of transposons.

There are 3 major roles of site-specific integration:

- Integration of a **fertility factor** to make an Hfr cell
- Integration of **temperate phage** DNA into a bacterial chromosome to create a prophage
- Movement and insertion of **transposons**

CONJUGAL CROSSES



1. In the male or F+ parent, the fertility factor is present but free from the bacterial chromosome. Transfer is uni-directional from male to female. *OriT*, as in every cross, will be transferred first and then the rest of the plasmid genes.

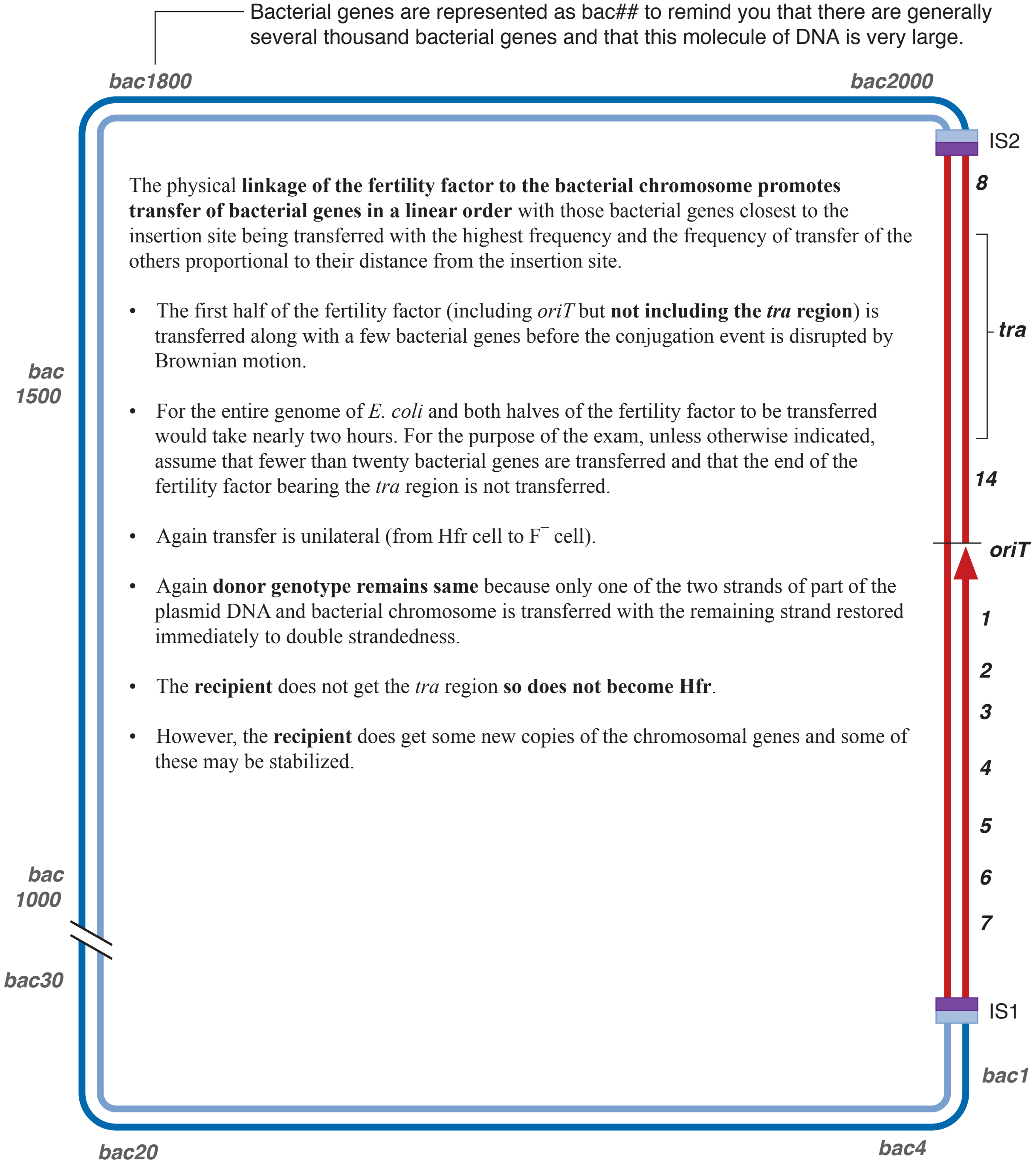
2. Only a single strand of the plasmid DNA duplex is transferred. The strand that is lost is reduplicated so that the donor always stays the same genotype. The last genes to be transferred are the *tra* region.

3. The transfer of the plasmid is fairly quick so assume it is transferred in its entirety 100% of the time unless otherwise told.

   Note that the F− cell undergoes a sex change, becoming F+ (male). These two F+ cells can no longer mate, and no BACTERIAL genes are transferred.

Figure II-3-7. F+ by F− Conjugal Cross

Integrated Fertility Factor



The physical **linkage of the fertility factor to the bacterial chromosome promotes transfer of bacterial genes in a linear order** with those bacterial genes closest to the insertion site being transferred with the highest frequency and the frequency of transfer of the others proportional to their distance from the insertion site.

- The first half of the fertility factor (including *oriT* but **not including the *tra* region**) is transferred along with a few bacterial genes before the conjugation event is disrupted by Brownian motion.
- For the entire genome of *E. coli* and both halves of the fertility factor to be transferred would take nearly two hours. For the purpose of the exam, unless otherwise indicated, assume that fewer than twenty bacterial genes are transferred and that the end of the fertility factor bearing the *tra* region is not transferred.
- Again transfer is unilateral (from Hfr cell to F^- cell).
- Again **donor genotype remains same** because only one of the two strands of part of the plasmid DNA and bacterial chromosome is transferred with the remaining strand restored immediately to double strandedness.
- The **recipient** does not get the *tra* region **so does not become Hfr**.
- However, the **recipient** does get some new copies of the chromosomal genes and some of these may be stabilized.

Figure II-3-8. Hfr Chromosome (Bacterial Chromosome with Integrated F Factor)

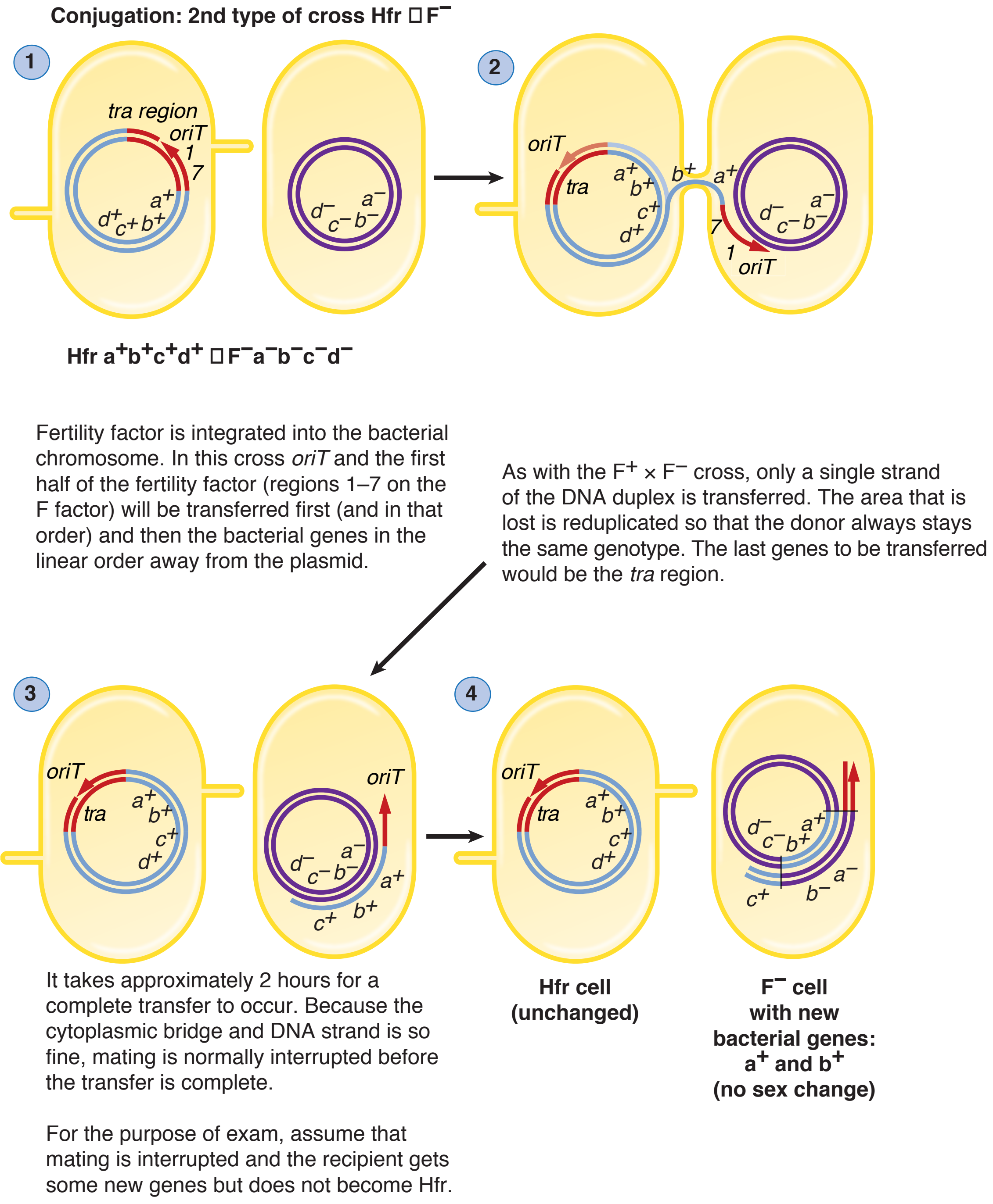


Figure II-3-9. Hfr by F⁻ Conjugal Cross

Transduction

Transduction is the **transfer of bacterial DNA by a phage vector**. During transduction, the phage picks up the bacterial DNA through an error in phage production.

- A **generalized transducing** phage is produced when the phage **with a lytic life cycle** puts a piece of bacterial DNA into its head. All bacterial genes have an equal chance of being transduced.
- **Specialized transduction** can occur when an error is made in the life cycle of a temperate (lysogenic) phage. Temperate phage introduce their genomic DNA into the bacterial chromosome at a specific site and then excise it later to complete their life cycle. If errors are made during the excision process, then bacterial chromosomal DNA can be carried along into the next generation of viruses.

In order to understand transduction, it is essential to understand what a bacteriophage is and how it replicates. A bacteriophage (sometimes called phage or bacterial virus) is **a virus that infects bacterial cells**.

There are 2 types of phage:

- **Virulent phage** infect bacterial cells, always making more virus and lysing the cells (lytic replication).
- **Temperate phage** often infect without lysing the cells because they have the ability to repress active phage replication and to stably integrate their DNA into the bacterial chromosome. In the absence of functional repressor protein, they also may replicate lytically.

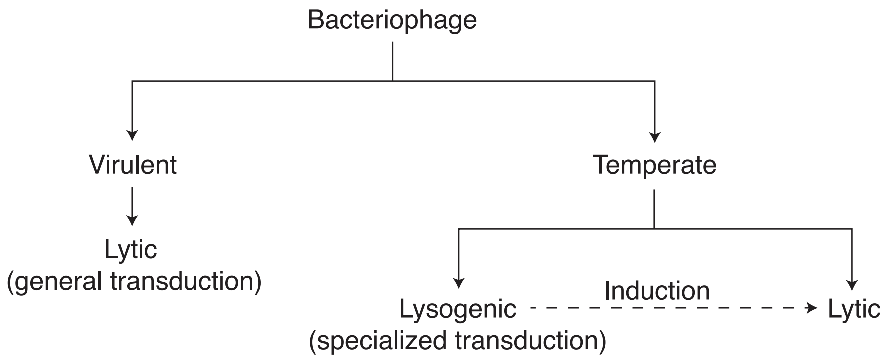


Figure II-3-10. Major Types of Bacteriophage

Lytic Infection

Lytic infection, by phage or viruses, leads to the production of viruses and their release by cell lysis.

- **Virulent viruses can only go into lytic life cycles and can accidentally carry out generalized transduction.**
- The lytic (or productive) life cycle of virulent phage is entirely normal except for a mistaken incorporation of bacterial DNA into one phage head, creating a transducing virus.

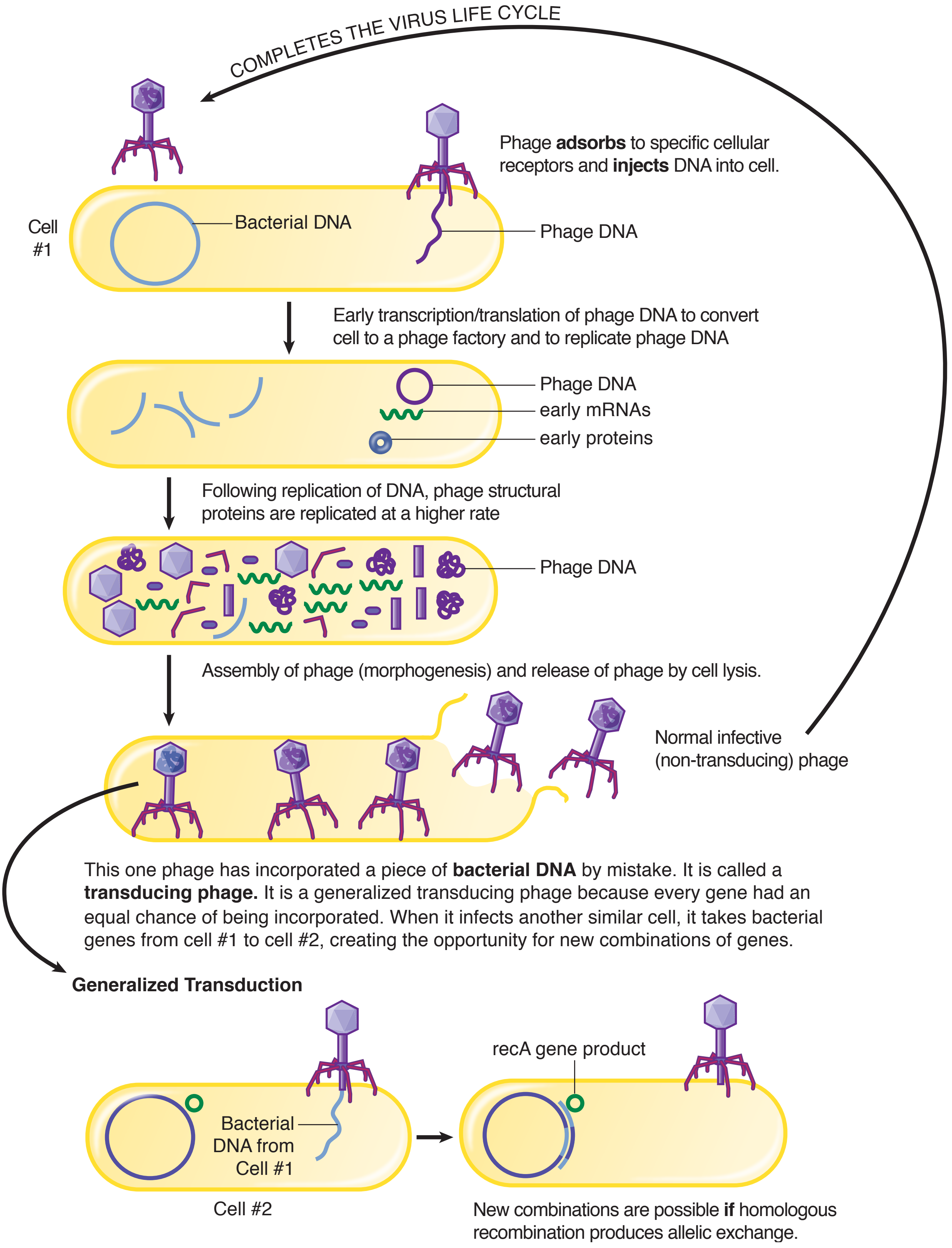


Figure II-3-11. Generalized Transduction as an Accident of Lytic Virus Life Cycle

Temperate Phage

Phage that have **both options (lytic replication or lysogeny)** are called **temperate phage**. When a temperate phage first infects a cell, there is a regulatory race which determines whether the repressor is made fast enough to prevent synthesis of phage components. The lysogenized cell will replicate to produce 2 identical cells, each with a prophage as long as the repressor gene product is present.

Lysogeny

Temperate phage may become prophage (DNA stably integrated) or replicate lytically.

- When repressor is made, temperate phage inserts its DNA into the bacterial chromosome where it stably stays as a **prophage**.
- Lysogeny is the state of a bacterial cell with a **stable phage DNA** (generally integrated into the bacterial DNA), **not undergoing lytic replication because it is repressed or defective**. When the cell DNA replicates, the phage DNA also replicates and, as long as the repressor protein is not damaged, the lysogenic state continues ad infinitum. Defective phage (or defective viruses in the human equivalent) cannot go into an active replication unless a helper virus is present.
- If the repressor gene gets mutated or the repressor protein gets damaged, then the prophage gets excised from the bacterial DNA and is induced into the lytic production of virus. On rare occasions, these temperate phage can produce either specialized or generalized transducing viruses. Lambda phage of *E. coli* is the best studied. Most temperate phages have only a single insertion site.
- Lambda inserts **ONLY between *E. coli* genes *gal* and *bio***.

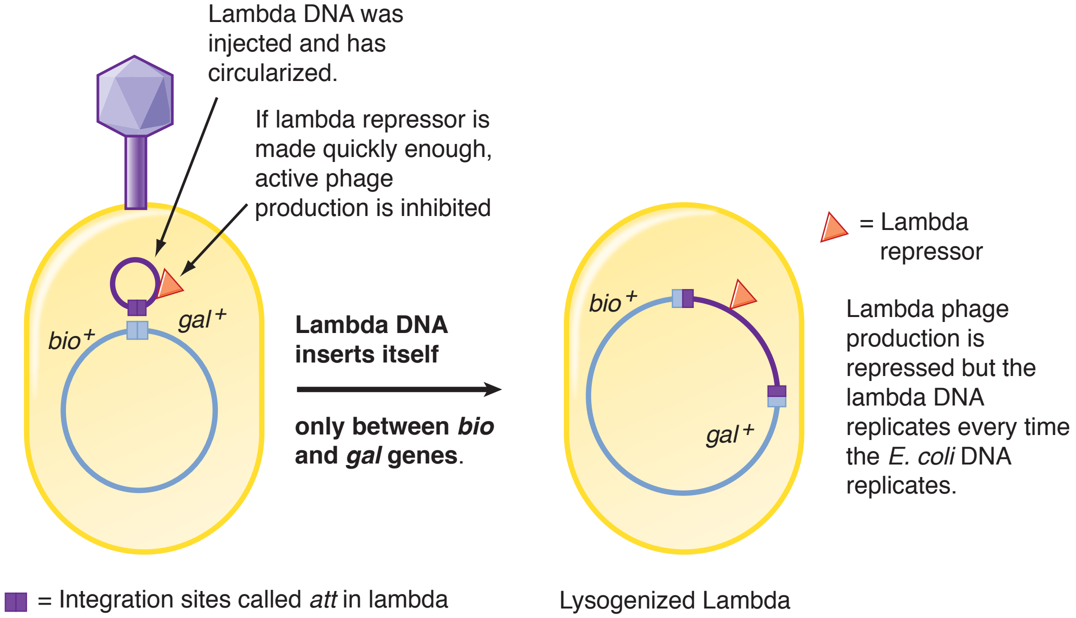


Figure II-3-12. Lysogeny

Lysogeny can confer new properties on a genus such as toxin production or antigens **(lysogenic conversion)**. It is a model for retrovirus provirus, allowing for specialized transduction.

To remember phage-mediated pathogenic factors, remember the mnemonic **COBEDS**:

- **C**holera toxin
- Presence of specific prophage in *Salmonella* can affect **O** antigens
- Phage CE β or DE β cause *Clostridium botulinum* to produce **b**otulinum toxin
- **E**xotoxins A–C (erythrogenic or pyogenic) of *Streptococcus pyogenes*
- Prophage beta causes *Corynebacterium diphtheriae* to make **d**iphtheria toxin
- **S**higa toxin

Induction

If the repressor is damaged (by UV, cold, or alkylating agents), then the prophage is excised and the cell goes into lytic replication phase. This process is called **induction**.

Most of the time the process is carried out **perfectly**, recreating the figure 8 of DNA that was the product of viral site-specific recombination, and normal **(nontransducing) phage** are produced.

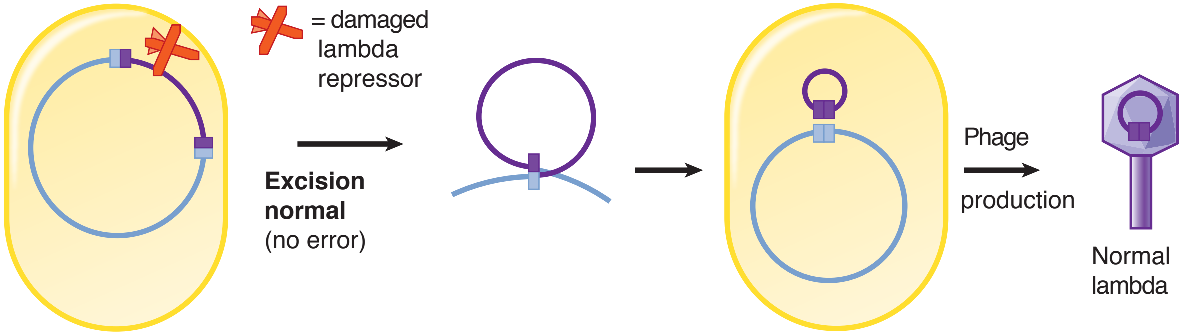


Figure II-3-13. Normal Excision of a Lysogenic Phage

Rarely, in the excision process, an **excisional error** is made and **one of the bacterial genes next to the insertion site is removed attached to the lambda DNA, and a little bit of lambda DNA is left behind**. Only genes on one side *or* the other side of the virus insertion site can be incorporated by excisional error.

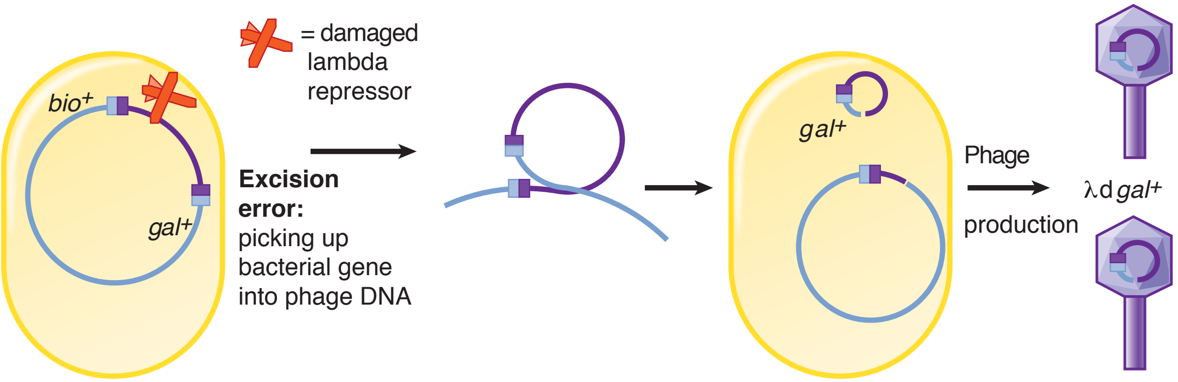


Figure II-3-14. Excision Error of a Lysogenic Phage

Because lambda has only one insertion site (between *gal* or *bio*), only *gal* or *bio* can be incorporated by excisional error.

Because all of the phage genes are still in the cell, phages are still made with the circular defective phage genome copied and put in each phage head. These are **specialized transducing phage** (only able to transduce *bio* or *gal*).

Specialized transduction occurs when bacterial genes picked up by error in the excision process are transferred to another closely related but often genetically distinct cell. If any genes on the exogenote are stabilized by recombinational exchange, then new genetic combinations occur.

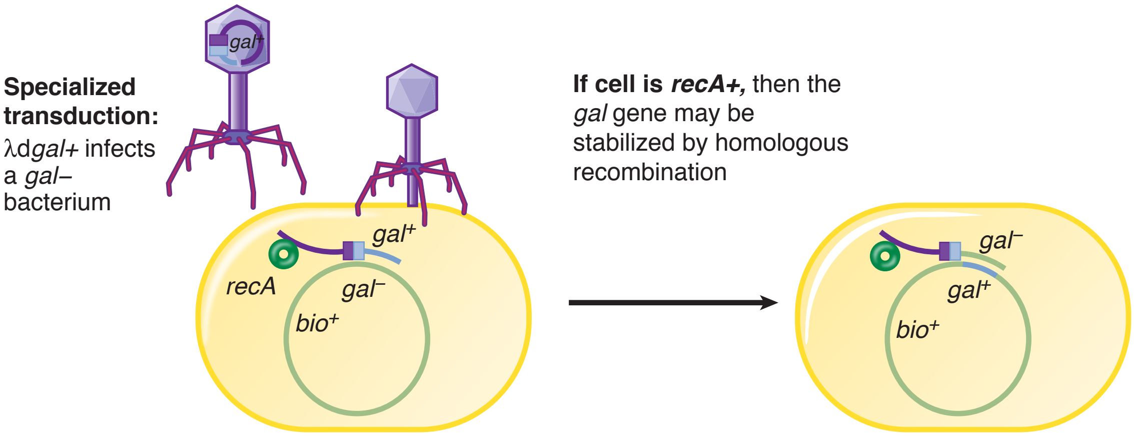


Figure II-3-15. Specialized Transduction

Only those genes next to the phage insertion site can be transduced by specialized transduction.

Table II-3-1. Transformation, Conjugation, and Transduction

Requirement	Transformation	Conjugation	Transduction
Is cell-to-cell contact required?	No	Yes	No
Does it require an antecedent phage infection?	No	No	Yes
Is competency required?	Yes	No	No
Is naked (free) DNA involved?	Yes	No	No
Is recombination required to stabilize new genes?	Yes	No for $F^+ \times F^-$ Yes for Hfr $\times F^-$	Yes

Table II-3-2. Generalized and Specialized Transduction

	Generalized	Specialized
Mechanism	**Error in assembly**	**Error of excision** Requires stable insertion of prophage DNA (lysogeny)
Genes that may be transferred	**Any**	Only **genes next to insertion site**

DRUG RESISTANCE

Drug resistance is becoming a significant problem. There are bacteria for which most antibiotics no longer work, and we are entering a "post-antibiotic era."

There are 3 types of antibiotic resistance: intrinsic, chromosome-mediated, and plasmid-mediated. Drug resistance can be transferred from one genus of bacteria to another, e.g., from normal flora to a pathogen.

Intrinsic Drug Resistance

Bacteria are intrinsically resistant to an antibiotic if they lack the target molecule for the drug or if their normal anatomy and physiology make them refractory to the drug's action.

- Bacteria that lack mycolic acids are intrinsically resistant to isoniazid.
- Bacteria such as *Mycoplasma* that lack peptidoglycan are intrinsically resistant to penicillin.

Chromosome-Mediated Resistance

In chromosome-mediated resistance, resistance is conveyed by genes located on the bacterial chromosome.

- Most commonly, these genes modify the **receptor for a drug** so the drug can no longer bind (e.g., a mutation in a gene for a penicillin-binding protein).
- In general, **drug resistance** is low level.

- In methicillin-resistant *Staphylococcus aureus*, a major penicillin-binding protein was mutated.
- Even low-level resistance may be clinically significant, e.g., in *Streptococcus pneumoniae* meningitis.

Plasmid-Mediated Resistance

The genes that determine this resistance are located on plasmids.

- Plasmid-mediated resistance is created by a variety of mechanisms, but often genes code for **enzymes that modify the drug**.
- R factors are conjugative plasmids carrying genes for drug resistance.
  - One section of the DNA (containing *oriT* and the *tra* gene region) mediates conjugation.
  - The other section **(R determinant)** carries genes for drug resistance. Multiple genes seem to have been inserted through **transpositional insertion** into a "hot spot."

Multiple drug-resistance (MDR) plasmids arise from mobile DNA segments known as **transposons (integrons)** or gene cassettes. Transposons have the following features:

- Are mobile genetic elements (DNA) that can move themselves or a copy from one molecule of DNA to another ("jumping genes")
- Are found in eukaryotic and bacterial cells and viruses
- Have at least 1 gene for a **transposase** (enzyme involved in the movement)
- Create additional **mutations** with their insertion into another totally unrelated gene

A typical genetic map of an R factor (conjugative drug-resistant plasmid) is shown below:

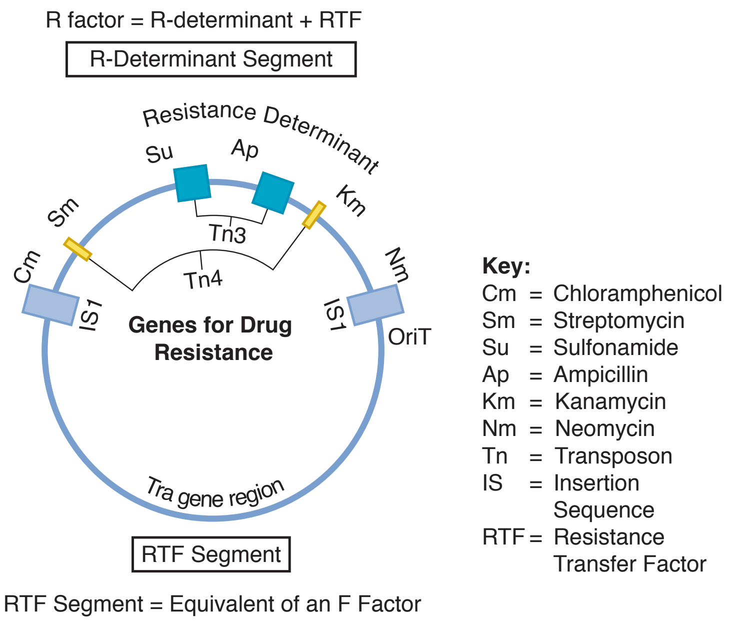


Figure II-3-16. Genetic Map of an R Factor

Table II-3-3. Plasmid-Mediated Mechanisms of Bacterial Drug Resistance

Antimicrobial Agent	Mechanism
Penicillins and cephalosporins	Production of β-**lactamase**; cleavage of β-lactam rings
Aminoglycosides	Production of acetyltransferase, adenosyltransferase, or phosphotransferase; **inactivation of drug by acetylation**, adenosylation, or phosphorylation
Chloramphenicol	Production of acetyltransferase; **inactivation of drug by acetylation**
Tetracyclines	Increased **efflux** out of cell
Sulfonamides	Active **export** out of cell and lowered affinity of enzyme
Vancomycin	Ligase produces cell wall pentapeptides that terminate in D-alanine-D-lactate, which will not bind to drug

Table II-3-4. Emerging Bacterial Resistances to Antimicrobial Agents

Antimicrobic	Mechanism		
Inhibitors of Cell Wall Synthesis			
	Altered Accumulation	**Altered Target**	**Enzymatic Inactivation**
β-lactams	Variable outer membranes (chromosome mediated): Gram (–), e.g., *Pseudomonas aeruginosa* (ceftazidime)	Mutant and new PBPs (chromosome mediated): *Streptococcus pneumoniae* (penicillin); *Haemophilus influenzae* (ampicillin); *Staphylococcus aureus* (methicillin); *Neisseria gonorrhoeae* (penicillin)	β-lactamases* (plasmid mediated), *Staphylococcus aureus* (penicillin); *Haemophilus influenzae* (ampicillin); *Neisseria gonorrhoeae* (penicillin); *Klebsiella* and *Enterobacter* spp. (third-generation cephalosporins)
Glycopeptides	Increased cell wall thickness (chromosome mediated), vancomycin intermediate *Staphylococcus aureus* (VISA)	Amino acid substitution (chromosome- or plasmid-mediated transposon), *Enterococcus faecalis* and *E. faecium* (vancomycin); *Staphylococcus aureus* (plasmid mediated), vancomycin, VRSA†	—
Isoniazid	—	—	Mutation of catalase-peroxidase gene needed to activate the drug (chromosome mediated), *Mycobacterium tuberculosis*
Ethambutol	—	Mutation of arabinosyl transferase gene (chromosome mediated), *Mycobacterium tuberculosis*	—

(Continued)

Table II-3-4. Emerging Bacterial Resistances to Antimicrobial Agents (*Cont'd*)

Antimicrobic	Mechanism		
Inhibitors of Protein Synthesis			
	Altered Accumulation	**Altered Target**	**Enzymatic Inactivation**
Aminoglycosides	Oxidative transport required (plasmid mediated), *Pseudomonas aeruginosa*, gentamicin	Ribosomal binding site mutations (chromosome mediated), *Enterococcus*, gentamicin	Adenylases, acetylases, phosphorylases (plasmid mediated), *Klebsiella* and *Enterobacter* spp., gentamicin
Macrolides, lincosamides	Minimal outer membrane penetration (chromosome mediated), Gram (–); efflux pump (plasmid mediated), Gram (+) cocci; erythromycin	Methylation of 23S rRNA (plasmid mediated), *Bacteroides fragilis*, *Staphylococcus aureus*, MLS resistance‡	Phosphotransferase, esterase (plasmid mediated), Gram (+) cocci
Chloramphenicol	—	—	Acetyltransferase (plasmid mediated), *Salmonella*, chloramphenicol
Tetracycline	Efflux pump (transposon in plasmid), widespread due to use in animal feed	New protein protects ribosome site (transposon in chromosome or plasmid), *Staphylococcus aureus*	—
Inhibitors of Nucleic Acid Synthesis			
Fluoroquinolones	Efflux pump (plasmid mediated), *Enterococcus*; permeability mutation (chromosomal), *Pseudomonas*	Mutant topoisomerase (chromosome mediated), *Escherichia coli* and *Pseudomonas aeruginosa*, ciprofloxacin	—
Rifamycins	—	Mutant RNA polymerase (chromosome mediated), *Mycobacterium tuberculosis*, *Staphylococcus* spp., and *Neisseria meningitidis*, rifampin	—
Folate inhibitors	—	New dihydropteroate synthetase, altered dihydrofolate reductase (chromosome mediated), Enterobacteriaceae sulfonamides	—

* Gram (+) β-lactamases are exoenzymes with little activity against cephalosporins, methicillin, or oxacillin. Gram (–) β-lactamases act in the periplasmic space and may have both penicillinase and cephalosporinase activity. Extended spectrum β-lactamases (ESBLs) are inducible and may not be detected by susceptibility testing. The range of ESBLs includes multiple cephalosporins. *TEM-1* is the most common of the plasmid-lactamase genes.

† The most common vancomycin resistance genes, *vanA* and *vanB*, are found in a transposon. These have been transferred from *Enterococcus* to a multidrug resistance plasmid in *Staphylococcus aureus*. The super multidrug resistance plasmid now contains resistance genes against-lactams, vancomycin, aminoglycosides, trimethoprim, and some disinfectants.

‡ MLS, macrolide-lincosamide-streptogramin resistance. Methylation of the 23S rRNA will impart resistance to erythromycin, lincomycin, and clindamycin.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Kirby-Bauer Agar Disk Diffusion Test

With this test, a solid medium with the patient's isolate is swabbed on the entire plate surface. Multiple paper disks, each with a single dried drug, are placed on the plate. The hydration and diffusion of the drug set up a concentration gradient during incubation and growth of the bacteria.

The diameter of the zones of inhibition must be measured to determine significance.

- **Advantages**: inexpensive, easy, can test numerous antibiotics on one plate, wealth of information based on clinical correlation
- **Disadvantages**: qualitative

Bacterial isolate is classified as resistant, intermediate, or susceptible to each drug.

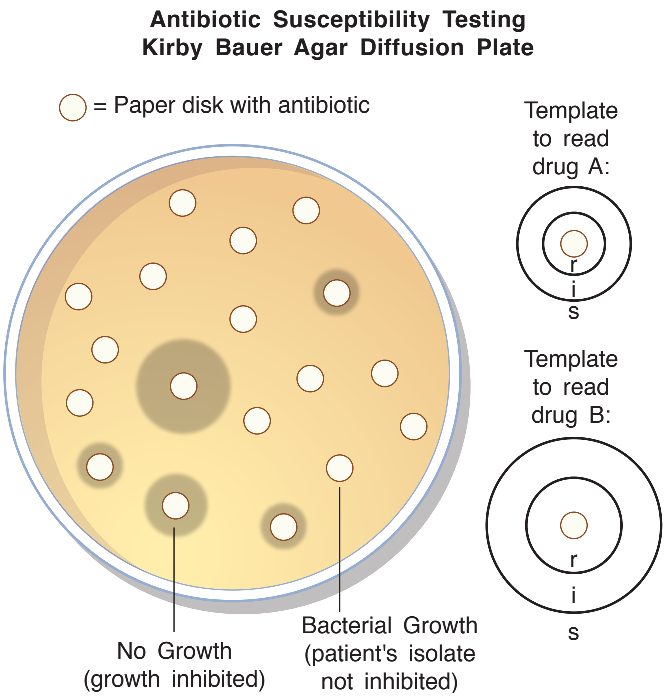


Figure II-3-17. Kirby-Bauer Agar Disk Diffusion Technique

Minimal Inhibitory Concentration

Minimal inhibitory concentration (MIC) measures antibiotic inhibition of bacterial multiplication. This is a dilution technique where **each container** (well of microtiter plate, test tube, or automated system bottle) **has one concentration of an antibiotic** with the patient's isolate. One control container always has just the patient's isolate and growth medium with no antibiotic, to make sure the inoculum is viable.

- **MIC is lowest concentration showing no visible growth**
- Indicates levels needed to inhibit; does not necessarily indicate killing levels (done with MBC)

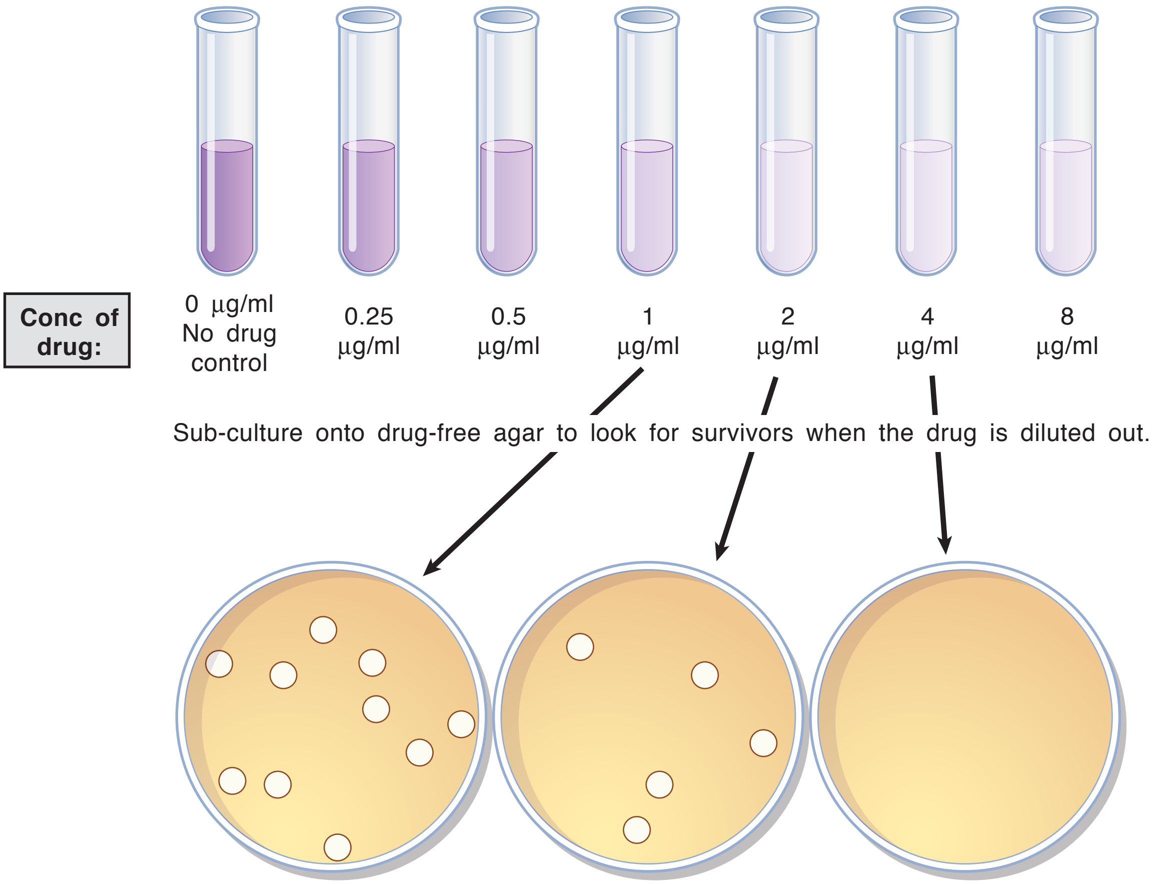
Minimal Bactericidal Concentration

Minimal bactericidal concentration (MBC) measures the antibiotic killing (bactericidal activity). This is a dilution technique starting with the MIC containers and subplating onto solid medium. Because a small inoculum is used on the plate with a large volume of medium, this dilutes the drug way below the MIC and allows determination of viability of cells.

- **MBC is lowest antibiotic concentration showing no growth on subculture to media without the antibiotic**
- Important for treating immunocompromised patients whose immune system cannot kill the bacteria while they are inhibited

Minimal Inhibitory Concentration = MIC

1. Each container has one concentration of a drug.
2. Each container has identical inoculum of the patient's bacterial isolate
3. Must run a no drug control.
4. Lowest concentration showing no visible growth = MIC
   (in example, MIC = 2 μg/ml)



Minimal Bactericidal Concentration (MBC)

(Not routinely done in many hospitals but ordered when necessary)

The lowest drug concentration showing no growth on sub-culture to media without drugs = MBC
MBC in example would be 4 μg/ml.

Figure II-3-18. MIC and MBC for a Drug

Medically Relevant Viruses 4

Learning Objectives

- ❑ Demonstrate understanding of structure, morphology, and replication of medically important viruses
- ❑ Answer questions related to patterns of infection, resistance, and treatment of DNA viruses, positive-stranded RNA viruses, negative-stranded RNA viruses, double-stranded RNA viruses, onco-viruses, and prions
- ❑ Explain information related to viral hepatitis

STRUCTURE AND MORPHOLOGY

DNA or RNA*	+	Structural proteins (capsomers, etc.)	=	Nucleocapsid	=	Naked capsid virus
Enzymes e.g., polymerase						

Nucleocapsid	+	Host membrane with viral-specified glycoproteins (critical for infectiousness of viral progeny)	=	Enveloped virus

Figure II-4-1. Basic Virion

Bridge to Biochemistry

Positive-sense = coding strand
Negative-sense = template strand

*Positive-sense RNA = (+)RNA (can be used itself as mRNA)

*Negative-sense RNA = (–)RNA

- Complementary to mRNA
- Cannot be used as mRNA
- Requires virion-associated, RNA-dependent RNA polymerase (as part of the mature virus)

VIRAL STRUCTURE

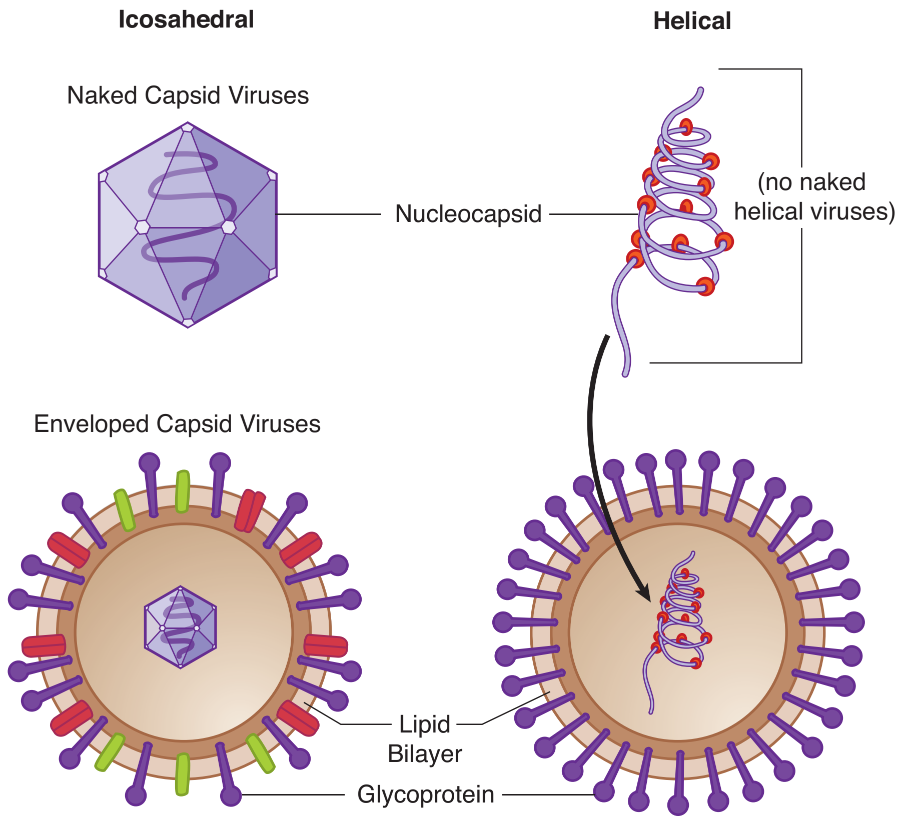


Figure II-4-2. Morphology of Viruses

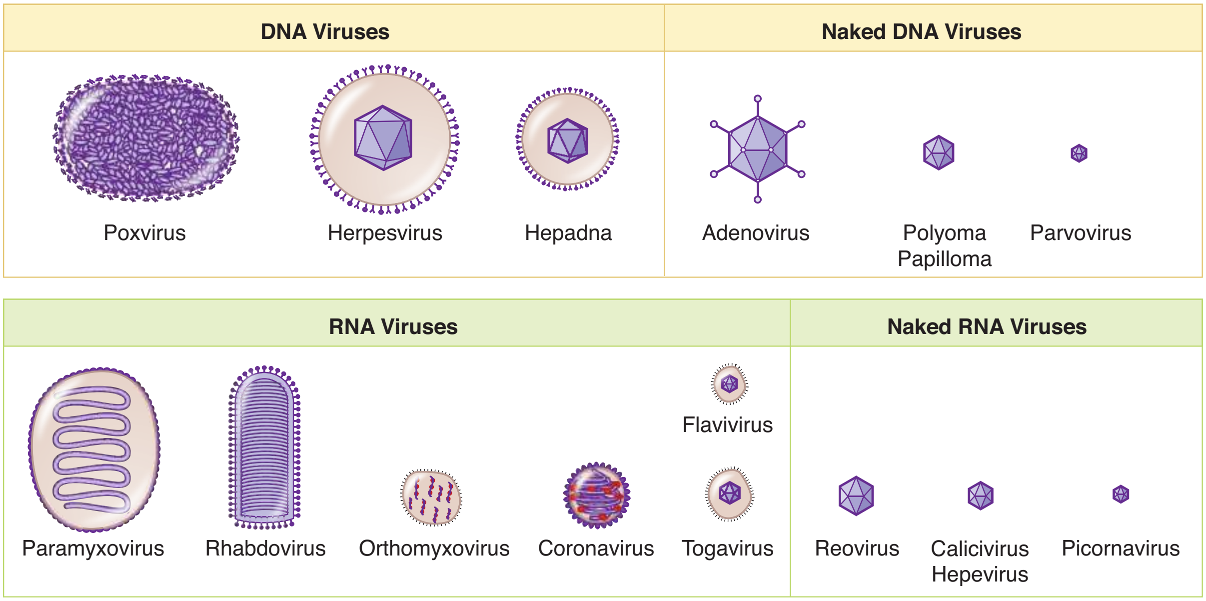


Figure II-4-3. Relative Sizes and Shapes of Different Viruses

VIRAL REPLICATION

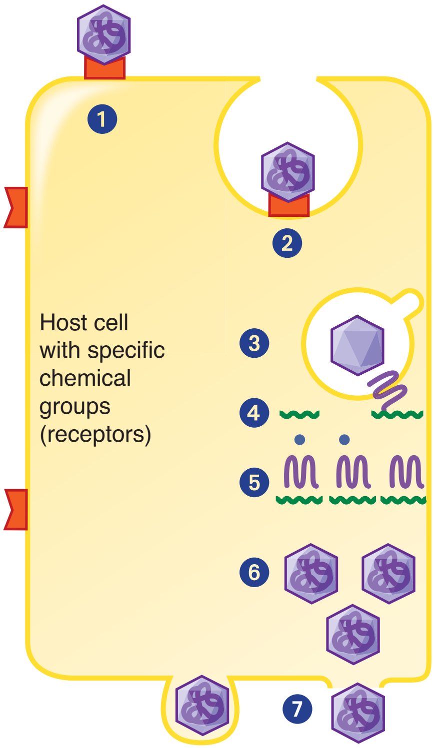


Figure II-4-4. Generalized Viral Replication Scheme

The following are drugs that target viral replication:

Number from Figure Above	Stages of Viral Replication	Examples of Drugs that Target	Mechanism of Action	Use to Treat
1	Attachment	Maraviroc	CCR5 antagonist	HIV
2	Penetration	Enfuvirtide	Fusion inhibitor	HIV
3	Uncoating (no longer used; only had activity against influenza A)	Amantadine/ rimantadine	Blocks uncoating via M2 protein	Influenza
4	Macromolecular synthesis (just examples; largest list of antivirals)	Acyclovir. zidovudine, etc.	Nucleic acid synthesis	Many
5	Post-translational modification of proteins	Ritonavir	Protease inhibitor	HIV
6	Assembly	N/A	N/A	N/A
7	Release	Oseltamivir	Neuraminidase inhibitor	Influenza
Retrovirus specific	Integration	Raltegravir	Integrase inhibitor	HIV

IMPORTANT STEPS IN VIRAL REPLICATION

Spread

Viruses are spread basically by the same mechanisms (e.g., respiratory droplets or sexually) as other pathogens. **Arthropod-borne** viruses are referred to as **arboviruses**.

Most belong to 3 formal taxonomic groups:

- Togavirus encephalitis viruses (a.k.a. alphaviruses)
- Flavivirus
- Bunyavirus

Mosquitoes are the most common vectors, while ticks, biting midges, and sand-flies are less common.

Attachment

Viruses bind through specific interaction with the host cell surface components **and**

- Specific **viral surface glycoproteins** of **enveloped viruses**, or
- Specific **viral surface proteins** of **naked viruses**.

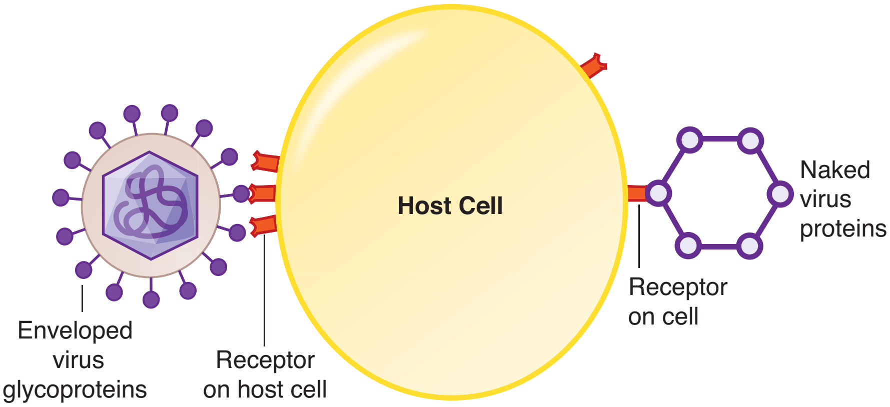


Figure II-4-5. Attachment

These interactions (and the distribution of the receptors) **determine viral host range** (e.g., horses or humans) and **tissue specificity** (e.g., liver versus heart; **tropism**).

Table II-4-1. Specific Viral Receptors to Know

Virus	Target Cell	Receptor on Host Cell
HIV	Th cells, macrophages, microglia	CD4 plus CCR5 or CXCR4
EBV	B lymphocytes	CD21 = CR2
Rabies	Neurons	Acetylcholine receptor
Rhinovirus	Respiratory epithelial cells	ICAM-1

Table II-4-2. Difference Between Naked and Enveloped Viruses

	Naked	Enveloped
Inactivated by heat, detergents, acid and organic solvents like ether and alcohols?	No	Yes, since the lipid envelope holds the glycoproteins essential for attachment. Dissolving the envelope inhibits attachment and therefore uptake.

Viral Entry Into Host Cell

Viral entry takes place by receptor-mediated endocytosis, uptake via coated pits, or for those enveloped viruses with fusion proteins via fusion of the cell membrane with the viral envelope.

Replication of the Genomic Nucleic Acid (NA)

Progeny viruses have a nucleic acid sequence **identical to the parent virus.** All single-stranded **RNA viruses replicate through a replicative intermediate**.

Table II-4-3. Strategy for Viral Genome Replication

Virus Type	Parental Genome	Intermediate Replicative Form	Progeny Genome
Most dsDNA viruses	dsDNA		dsDNA
Hepatitis B	dsDNA	ssRNA →	dsDNA
Most +ssRNA viruses	+ssRNA	−ssRNA	+ssRNA
Retroviruses	+ssRNA →	dsDNA	+ssRNA
−ssRNA viruses	−ssRNA	+ssRNA	−ssRNA

+ means an RNA which can serve as mRNA (or for the retroviruses has the same sequence.)
→ = RNA-dependent DNA polymerase

- Called reverse transcriptase for the retroviruses.
- Called the DNA polymerase for hepatitis B.
- Both actually make the first strand of the DNA using the RNA original and then break down the RNA and use the single strand of DNA as template to make the second strand.

Release of Viruses

Naked viruses lyse the host cells. Thus, there are **no persistent productive infections** with naked viruses (only cytolytic productive or latent infections).

Release of enveloped viruses: Budding leads to cell senescence (aging), but cells may produce a low level of virus for years as occurs in chronic hepatitis B.

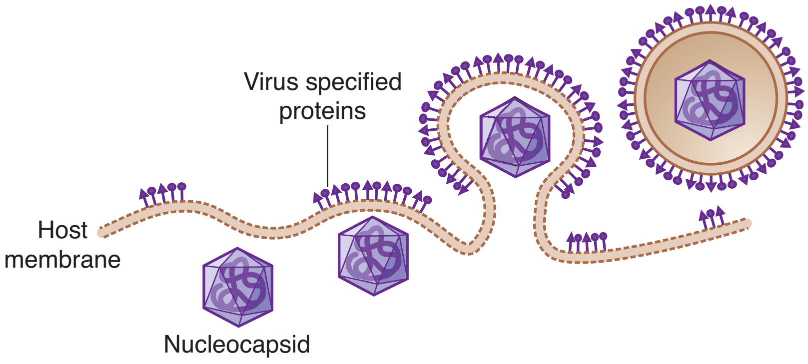


Figure II-4-6. Release of Enveloped Virus

The glycoproteins on the enveloped viral surface are essential for viral infectivity.

PATTERNS OF VIRAL INFECTION

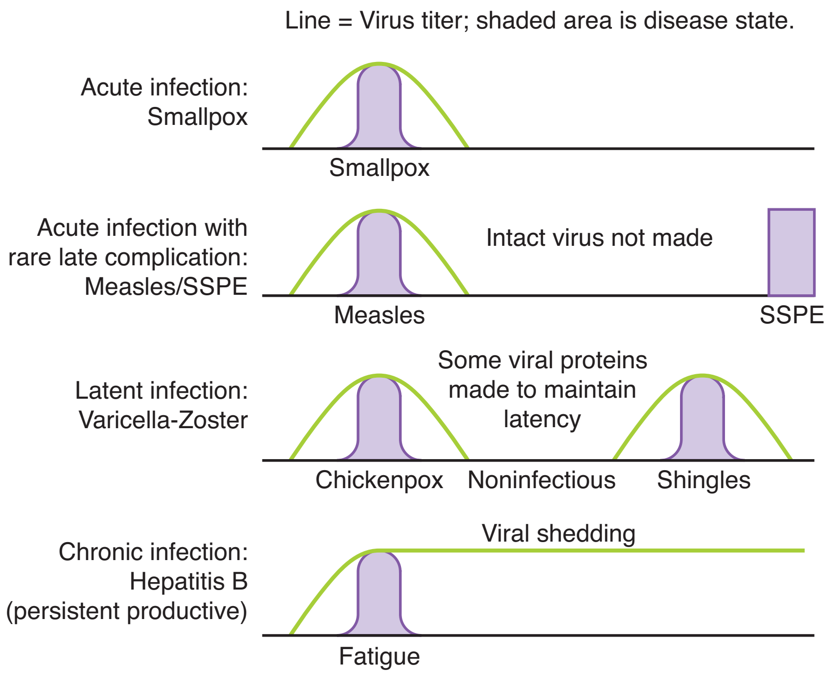


Figure II-4-7. Time Courses of Viral Infections

Table II-4-4. Cellular Effects

Infection Type	Virus Production	Fate of Cell
Abortive	–	No effect: No virus is made nor is latency established; Virus is terminated
Cytolytic Naked viruses lyse host cells. Some enveloped viruses also are cytolytic, killing the cell in the process of replication.	+	Lysis of the host cell (death)
Persistent Productive (enveloped viruses) Latent Transforming	 + – ±	 Senescence (premature aging) No overt damage to host; no production of virus, but viral production may be turned on later. Immortalization

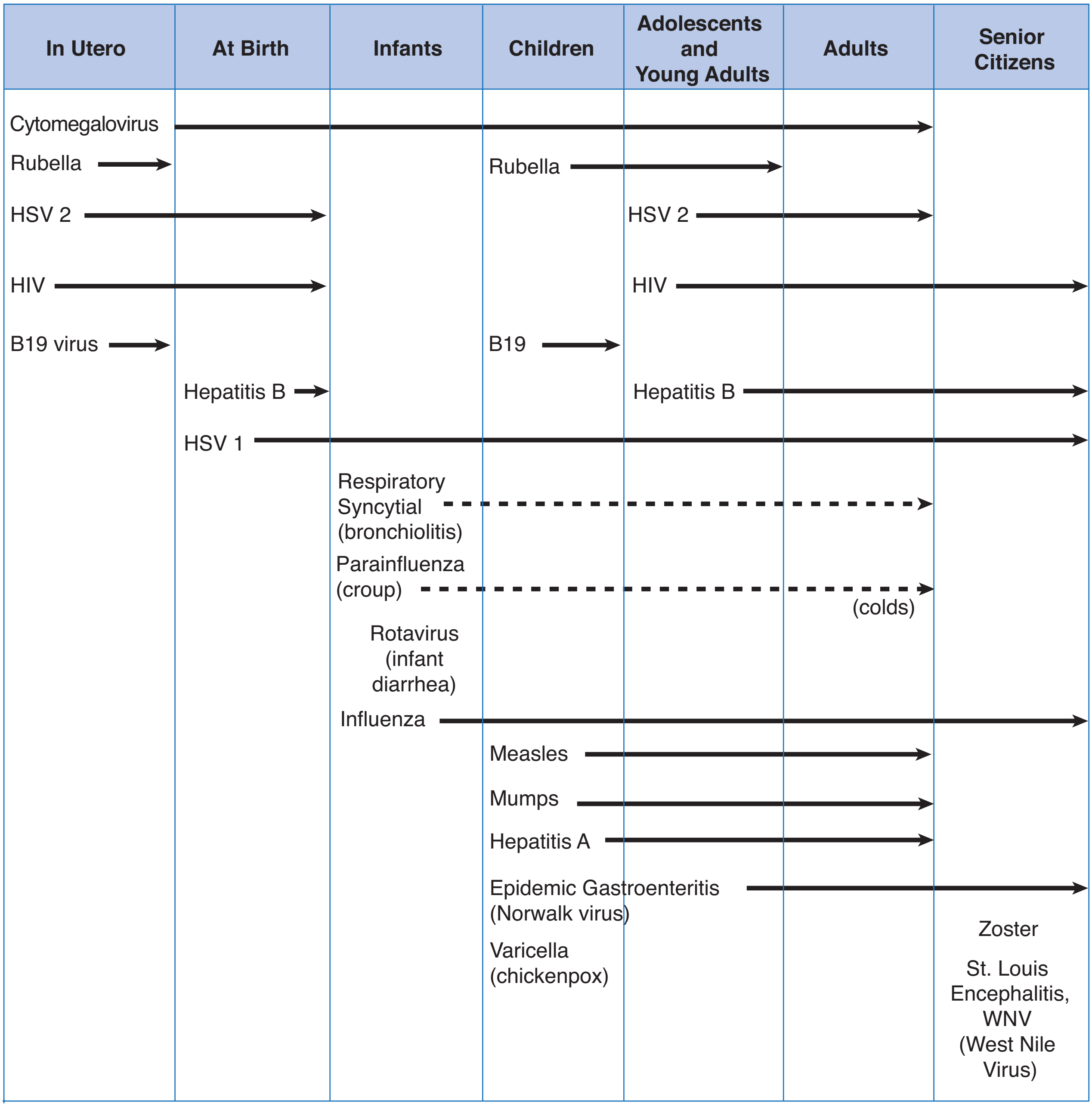


Figure II-4-8. Most Common Age Groups for Viral Infection

VIRAL HEPATITIS

Symptoms of Hepatitis

Fever, malaise, headache, anorexia, vomiting, dark urine, jaundice.

Table II-4-5. Hepatitis Viruses (Hepatotropic)

	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
	"Infectious" (HAV)	"Serum" (HBV)	"Post-transfusion Non A, Non B" (HCV)	"Delta" (HDV)	"Enteric" (HEV)
Family	Picornavirus	Hepadnavirus	Flavivirus	N/A	Hepevirus
Features	• RNA • Naked Capsid	• DNA • Enveloped	• RNA • Enveloped	• Viroid • Circular RNA • Enveloped	• RNA • Naked capsid
Transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Disease presentation	• Mild acute • No chronic • No sequelae	• Acute; occasionally severe • Chronic: 5–10% adults 90% infants • Primary hepatocellular carcinoma, cirrhosis	• Acute is usually subclinical • 80% become chronic • Primary hepatocellular carcinoma, cirrhosis	• Co-infection with HBV: occasionally severe • Superinfection with HBV: often severe • Cirrhosis, fulminant hepatitis	• Normal patients mild • Pregnant patients severe • Chronic in IC patients
Mortality	$<$0.5%	1–2%	0.5–1%	High to very high	• Normal patients 1–2% • Third-trimester pregnant patients 25%
Diagnosis	IgM to HAV	HBsAg, IgM to HBcAg	Antibody to HCV, ELISA	Hepatitis D Ab, HBsAg	Antibody to HEV, ELISA
Treatment	Symptomatic	IFN-α+RTI (NT analogs)	• Elbasvir/ grazoprevir* • Ledipasvir/ sofosbuvir	N/A	Mostly symptomatic**

*ribavirin, ±pegylated IFN-α for patients with cirrhosis

**ribavirin and pegylated IFN-α can be used for chronic

Note: Hepatitis also may occur in other viral diseases (e.g., CMV and EBV infections, congenital rubella, yellow fever).

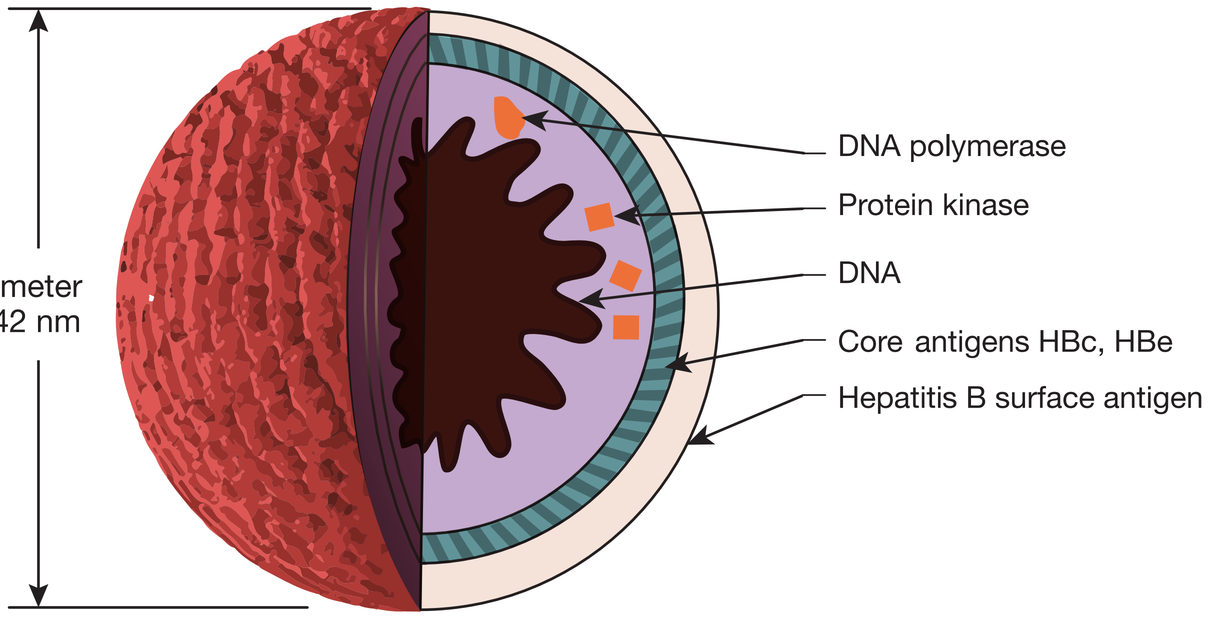


Figure II-4-9 Dane Particle

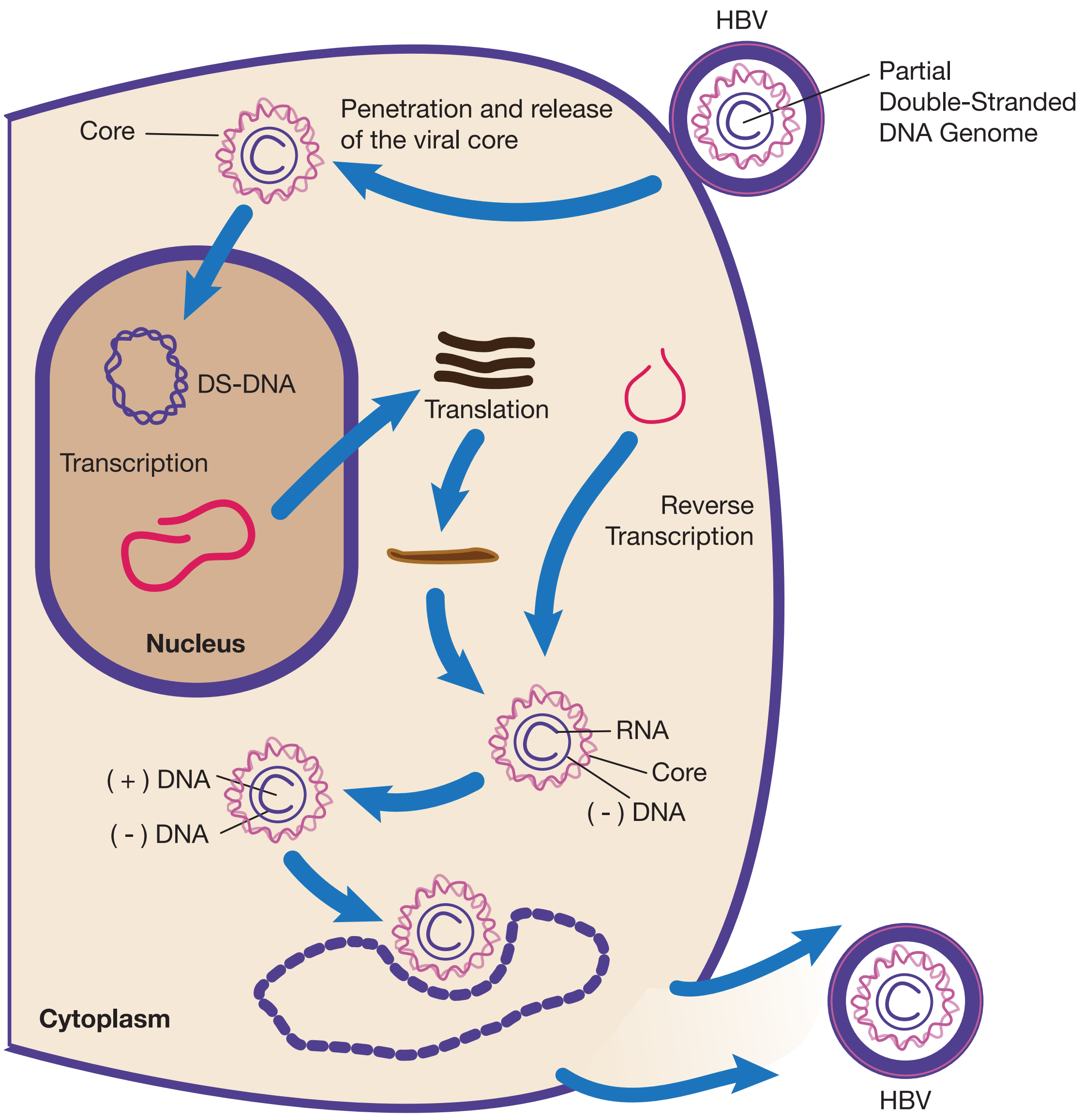


Figure II-4-10 Hepatitis B Virus Replication

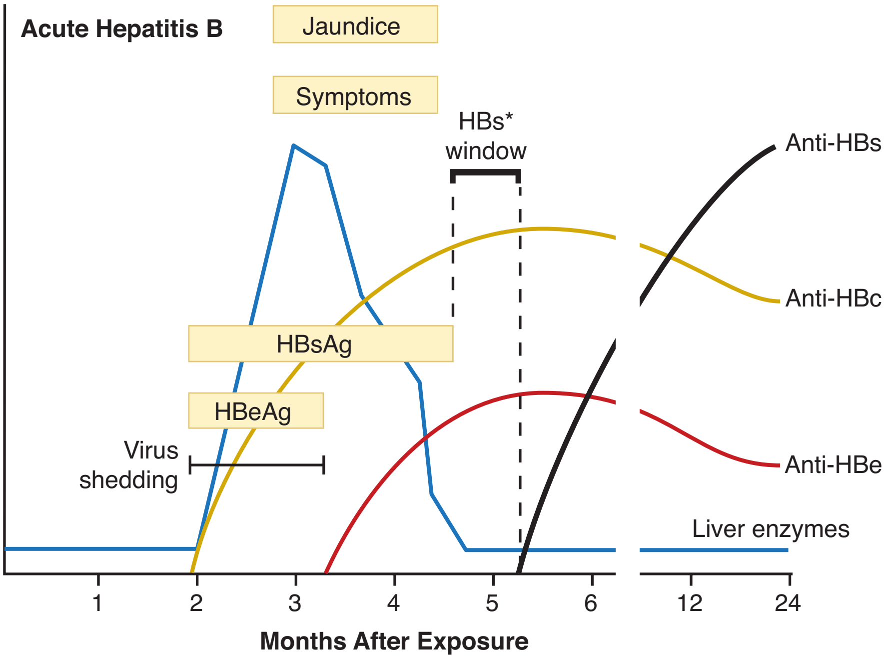
Table II-4-6. Hepatitis B Terminology and Markers

Abbreviation	Name and Description
HBV	Hepatitis B virus, a hepadnavirus (enveloped, partially double-stranded DNA virus); Dane particle = infectious HBV
HBsAg	Antigen found on surface of HBV; also found on spheres and filaments in patient's blood: positive during acute disease; continued presence indicates carrier state
HBsAb	Antibody to HBsAg; provides immunity to hepatitis B
HBcAg	Antigen associated with core of HBV
HBcAb	Antibody to HBcAg; positive during window phase; IgM HBcAb is an indicator of recent disease
HBeAg	A second, different antigenic determinant on the HBV core; important indicator of transmissibility
HBeAb	Antibody to e antigen; indicates low transmissibility
Delta agent	Small RNA virus with HBsAg envelope; defective virus that replicates only in HBV-infected cells
Window period	Period between end of detection of HBsAg and beginning of detection HBsAb

Table II-4-7. Hepatitis B Serology

	HBsAg HBeAg* HBV-DNA	HBcAb IgM	HBcAb IgG	HBeAb	HBsAb
Acute infection	+	+	–	–	–
Window period	–	+/–	+	+	–
Prior infection	–	–	+	+	+
Immunization	–	–	–	–	+
Chronic infection	+	–	+	+/–	–

*HBeAg: correlates with viral proliferation and infectivity



*The window is the time between the disappearance of the HBsAg and before HBsAb is detected.

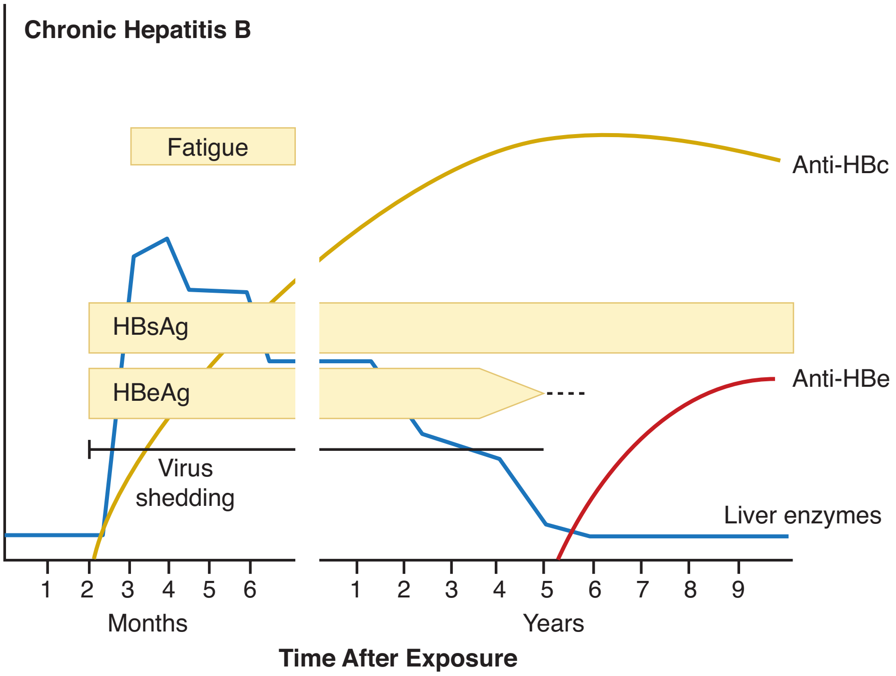


Figure II-4-11. Hepatitis B

DNA VIRUSES: CHARACTERISTICS

All DNA viruses are **double-stranded** (except parvovirus); are **icosahedral** (except poxvirus which is a brick-shaped "complex"); and **replicate their DNA in the nucleus** (except poxvirus).

Table II-4-8. DNA Viruses*

Virus Family	DNA type	Virion-Associated Polymerase	Envelope	DNA Replicates in:	Major Viruses
Parvovirus	ssDNA	No	Naked	Nucleus	B19
Papillomavirus Polyomavirus	dsDNA, circular	No	Naked	Nucleus	Papilloma, Polyoma
Adenovirus	dsDNA, linear	No	Naked	Nucleus	Adenoviruses
Hepadnavirus	Partially dsDNA, circular	Yes***	Enveloped	Nucleus, RNA intermediate	Hepatitis B
Herpes virus	dsDNA, linear	No	Enveloped (nuclear)	Nucleus; virus assembled in nucleus	HSV, Varicella-zoster, Epstein-Barr, Cytomegalovirus
Poxvirus	dsDNA, linear	Yes**	Enveloped	Cytoplasm	Variola, Vaccinia, Molluscum contagiosum

* Mnemonic: Pardon Papa As He Has Pox

** **Poxviruses** have a **virion-associated transcriptase** (DNA dependent RNA polymerase), so it can transcribe its own DNA in the cytoplasm and make all of the enzymes and factors necessary for replication of the poxvirus DNA in the cytoplasm.

*** Hepadnaviruses: DNA viruses that carry a DNA polymerase with reverse transcriptase activity to synthesize an RNA intermediate that is then used to make the genomic DNA. Hepatitis B is partially double-stranded with one complete strand.

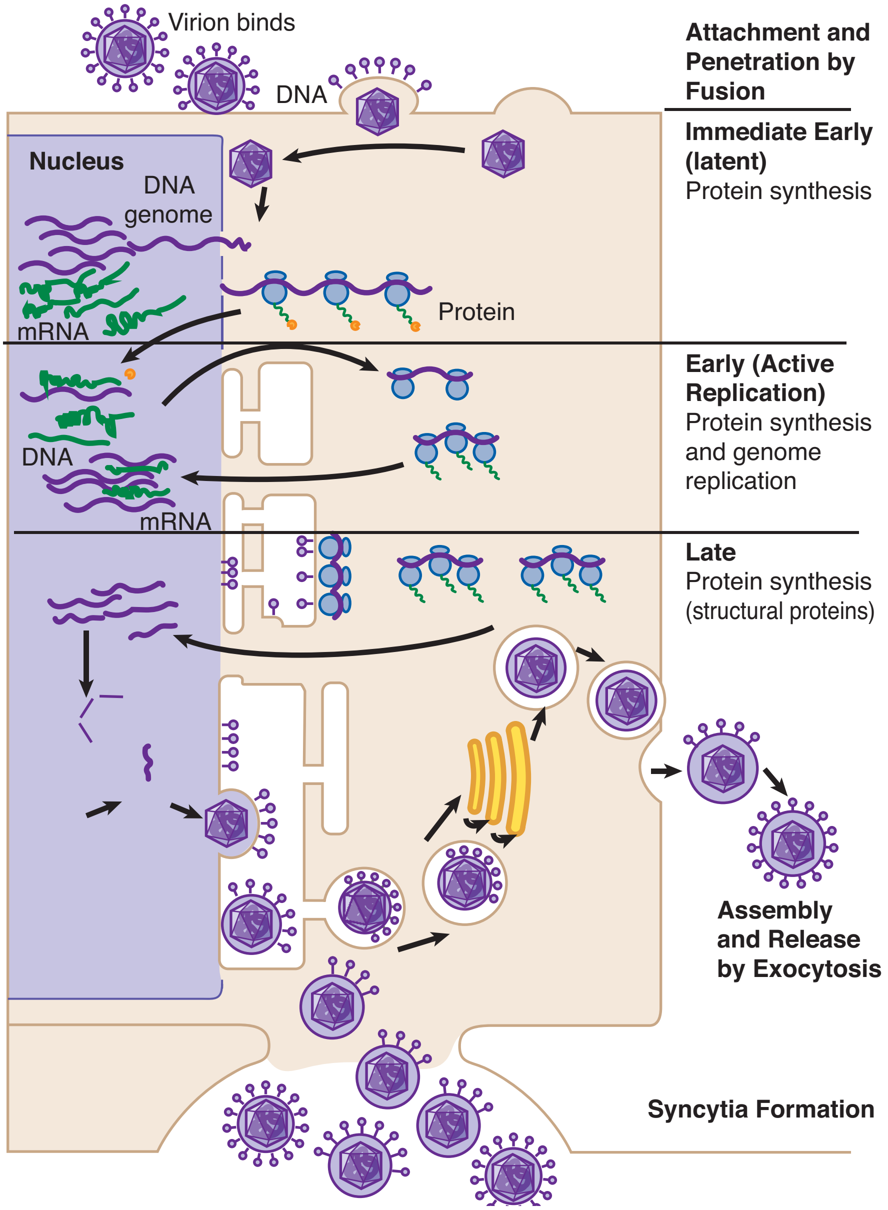


Figure II-4-12. DNA Virus: Life Cycle of Herpes

PARVOVIRIDAE

Virus Characteristics

- ssDNA virus, linear
- Naked, icosahedral

Viruses of Medical Importance: B19

Figure II-4-13. Parvovirus

B19

Reservoir: human respiratory tract

Transmission: respiratory route, fomites, vertical transmission

Pathogenesis: B19 infects immature (cycling) erythroid progenitor cells, causing cell lysis; the resulting **anemia** is only clinically significant in patients with **sickle-cell anemia**, and may result in **aplastic crisis**

Diseases

- Children/adults:
  - Fifth disease, erythema infectiosum, slapped cheek fever: 7–10 day incubation; nonspecific “flu-like” symptoms followed by raised, indurated facial rash; rash and arthralgias (mostly adults) are due to immune complexes in skin and joints
  - Myocarditis
- Fetus
  - Severe anemia
  - Congestive heart failure
  - Hydrops fetalis
  - Spontaneous abortion

Diagnosis: serology and molecular analysis

Treatment: supportive care

Key Vignette Clues

B19

- School-aged child with fever and indurated facial rash
- Pregnant woman with flu-like symptoms → hydrops fetalis or spontaneous abortion

PAPILLOMAVIRIDAE

Virus Characteristics

- dsDNA virus, circular
- Naked, icosahedral

Viruses of Medical Importance

- Human papilloma virus (HPV)

Figure II-4-14. Papillomavirus

Human Papilloma Virus (HPV)

Distinguishing Characteristics

- Over 100 serotypes
- Different serotypes are associated with different clinical presentations

Reservoir: human skin and genitals

Transmission: direct contact, fomites

Pathogenesis

- Virus infects basal layer of the skin and mucous membranes
- Hyperkeratosis leads to the formation of the "wart"
- Malignancy may result: **E6 and E7 inhibit tumor-suppressor genes p53 and Rb, respectively.**

Diseases

- Cutaneous warts
  - **Common warts** (serotypes 2 and 4) are predominantly found on hands and fingers
  - **Plantar warts** (serotype 1) are predominantly found on soles of feet; tend to be deeper and more painful
- Anogenital warts (Condylomata acuminata)
  - **Over 90% are serotypes 6 and 11 (benign)**
  - Also cause laryngeal papillomas in infants and sexually active adults
  - **Serotypes 16 and 18** are preneoplastic (cervical intraepithelial neoplasia or CIN), **31, 33, 35, 45, 52, 58** (16 and 18 >70% of cases)

Key Vignette Clues

HPV

- Warts
- Cervical intraepithelial neoplasia
- Biopsy or Pap smear—koilocytic cells

- Malignancy
  - Viral genes E6 and E7 inactivate tumor-suppressor genes
  - 95% of CIN cases contain HPV DNA
  - Also cause anal, vaginal, vulvar, penile, and laryngeal cancer

Diagnosis

- Cutaneous: clinical grounds
- Genital: finding of koilocytic cells (cells with perinuclear cytoplasmic vacuolization and nuclear enlargement) in Pap smear
- In situ DNA probes and PCR can be used to confirm any diagnosis and type the HPV strain involved

Treatment

- Antiviral: cidofovir
- Surgical: cryotherapy, laser therapy, excisional
- Chemical: podophyllin, trichloroacetic acid, 5-fluorouracil
- Immune-mediated: imiquimod, interferon-alpha
- Recurrence rates 30–70% within 6 months

Prevention: safe sex; vaccines composed of HPV capsid proteins produced by recombinant DNA technology: Gardasil™ quadrivalent (6, 11, 16, 18), Gardasil™ 9 (6, 11, 16, 18, 31, 33, 45, 52, 58), Cervarix™ (16, 18)

POLYOMAVIRIDAE

Table II-4-9. Summary of Polyomaviridae

Virus	Reservoir/ Transmission	Pathogenesis	Disease	Diagnosis	Treatment
BK	Respiratory	Latent infection in kidney	Renal disease in AIDS patients	ELISA, PCR	Supportive
JC	Respiratory	Infection in oligodendrocytes = demyelination	Progressive multifocal leukoencephalopathy (PML) in AIDS and transplant patients	ELISA, PCR	Supportive

ADENOVIRIDAE

Virus Characteristics

- dsDNA, nonenveloped
- Hexons, pentons, and fibers

Viruses of Medical Importance

- Adenovirus
- Over 50 serotypes
- Subgroups A–F

Adenovirus

Reservoir: ubiquitous in humans and animals

Transmission: respiratory, fecal-oral, direct contact

Pathogenesis

- Penton fibers act as hemagglutinin
- Purified penton fibers are toxic to cells
- **Lytic, latent**, or **transforming**: virus is lytic in permissive cells and can be chronic or oncogenic in nonpermissive hosts; the adenoviruses are standard example of permissive host (where virus is produced) and nonpermissive host (where virus is not produced but transformed)

Disease

- Acute respiratory disease (ARD) and pneumonia: spring and winter peak incidence; children, young military recruits, college students serotypes 4 and 7; cough, conjunctivitis, fever, pharyngitis, hoarseness
- Pharyngoconjunctivitis: swimming pool conjunctivitis, pink eye; fever, sore throat, coryza, red eyes; nonpurulent
- Acute hemorrhagic cystitis: mostly boys age 5–15; dysuria, hematuria
- Gastroenteritis: daycare (not as common as rotavirus); serotypes 40 and 41
- Myocarditis
- Transplant patients

Diagnosis: serology; ELISA

Treatment: supportive care for otherwise healthy patients; cidofovir and alpha globulins for immunocompromised or severely diseased

Prevention: live, nonattenuated vaccine

Key Vignette Clues

Adenovirus

- Young adults: ARD
- Swimmers and shipyard workers: nonpurulent conjunctivitis
- Daycare: viral gastroenteritis

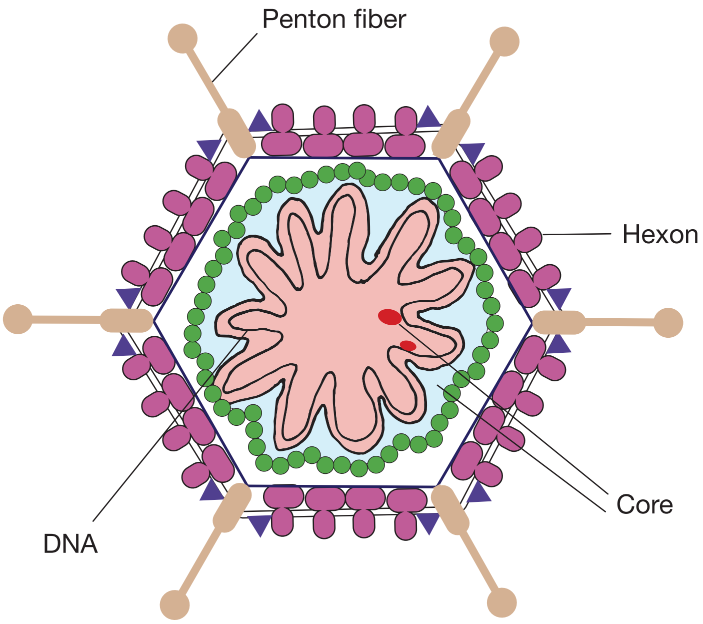


Figure II-4-15. Adenovirus

HEPADNAVIRIDAE (see previous discussion)

HERPESVIRIDAE

Virus Characteristics

- Large dsDNA
- Enveloped, icosahedral
- Derives envelope from nuclear membrane
- **Intranuclear inclusion bodies**
- **Establishes latency**

Viruses of Medical Importance

- Herpes simplex virus 1 and 2 (HSV)
- Varicella-zoster virus (VZV)
- Epstein-Barr virus (EBV)
- Cytomegalovirus (CMV)
- Human herpesvirus 6 (HHV-6)
- Human herpesvirus 8 (HHV-8)

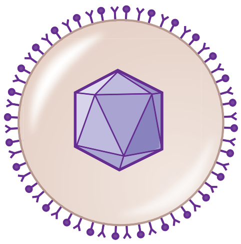


Figure II-4-16. Herpesvirus

HSV-1 and HSV-2

Reservoir: human mucosa and ganglia

Transmission: close personal contact (i.e., kissing, sexual contact)

Pathogenesis: HSV establishes infection in the mucosal epithelial cells and leads to formation of vesicles; virus travels up the ganglion to establish lifelong latent infection; stress triggers reactivation of virus in nerve and recurrence of vesicles

Diseases: Rule of thumb is that **HSV-1** infections generally **occur above the waist** and **HSV-2** infections generally **occur below the waist.**

HSV-1

- Gingivostomatitis and cold sores: blister-like lesions on oral mucosa; **latent in trigeminal ganglion**
- Keratoconjunctivitis: generally with lid swelling and vesicles; possible dendritic ulcers; if untreated and repeat attacks, possible blindness
- Encephalitis: fever, headache, confusion; **focal temporal lesions** and perivascular cuffing; if untreated, 70% mortality rate; **most common cause of viral encephalitis in U.S.**

HSV-2

- Genital infections: painful genital vesicles; systemic effects include fever, malaise, myalgia; **latency in sacral nerve ganglia**
- Neonatal herpes: infection occurs during passage through infected birth canal; usually **severe**, i.e., disseminated with liver involvement and high mortality; encephalitis, high mortality; skin, eyes, mouth)

Diagnosis

- Oral lesions: clinical
- Encephalitis: PCR on CSF; large numbers of RBCs in CSF
- Genital infections: Tzank smear to show formation of multinucleated giant cells and Cowdry type A intranuclear inclusions has been largely replaced by immunofluorescent staining, which can distinguish HSV-1 from HSV-2

Key Vignette Clues

HSV-1 and HSV-2

- Cold sores/genital vesicles
- Keratoconjunctivitis
- Meningoencephalitis/encephalitis
- Neonatal disseminated/encephalitis
- Tzanck smear, Cowdry type A inclusion bodies

Treatment: Acyclovir, a nucleoside analog that is only activated in cells infected with HSV-1, HSV-2 or VZV. (This is because the virus thymidine kinase is required to activate the drug by placing the first phosphate on the drug, followed by the phosphorylation via cellular enzymes.) In cases of acyclovir resistance (caused by a mutation in the thymidine kinase), use famciclovir, valacyclovir, or penciclovir.

Key Vignette Clues

VZV

- Chickenpox: unvaccinated child with asynchronous rash
- Shingles: elderly with unilateral vesicular rash that follows dermatome
- Tzank smear with Cowdry type A intranuclear inclusions and syncytia

Varicella Zoster Virus (VZV)

Reservoir: human mucosa and nerves

Transmission: respiratory droplets

Pathogenesis: VZV enters the respiratory tract → replicates in local lymph nodes → primary viremia → spleen and liver → secondary viremia → skin (rash) → **latent in the dorsal root ganglia.** Reactivation of virus due to stress or immunocompromise causes vesicular lesions and severe nerve pain.

Diseases

- Chickenpox
  - Fever, pharyngitis, malaise, rhinitis
  - **Asynchronous rash**
  - One of 5 "classic" childhood exanthems; less common due to vaccination
- Shingles
  - Zoster
  - Pain and vesicles restricted to one dermatome
  - Fifth or sixth decade of life
  - **Reactivation of latent infection**

Diagnosis

- Tzanck smear—Cowdry type A, intranuclear inclusions
- Antigen detection by PCR

Treatment

- Healthy adults with shingles—oral acyclovir
- Immunocompromised—IV acyclovir
- Aspirin contraindicated due to association with Reye syndrome

Prevention

- Live, attenuated vaccine, booster for 60-year-old to prevent shingles
- VZIG (varicella-zoster immunoglobulin) for postexposure prophylaxis of the immunocompromised

Epstein-Barr Virus (EBV)

Reservoir: humans

Transmission: saliva, 90% of adult population is seropositive

Pathogenesis

- Virus infects nasopharyngeal epithelial cells, salivary and lymphoid tissues → latent infection of B cells (EBV binds to CD21 and acts as a B-cell mitogen) → results in production of atypical reactive T cells (Downey cells), which may constitute up to 70% of WBC count
- Heterophile antibodies are produced (due to B cell mitogenesis)

Diseases

- Heterophile-positive mononucleosis, "kissing disease": fatigue, fever, sore throat, lymphadenopathy, splenomegaly; **latency in B cells**
- Lymphoproliferative disease: occurs in immunocompromised patients; T cells can't control B-cell growth
- Hairy oral leukoplakia: hyperproliferation of lingual epithelial cells; occurs in AIDS patients

Malignancies

- Burkitt lymphoma: cancer of the maxilla, mandible, abdomen; Africa; malaria cofactor; AIDS patients; translocation juxtaposes c-myc oncogene to a very active promoter such as immunoglobulin gene promoter
- Nasopharyngeal carcinoma: Asia (most common cancer in southern China); tumor cells of epithelial origin
- Hodgkin and non-Hodgkin lymphoma

Diagnosis: heterophile-antibody positive (IgM antibodies that recognize Paul-Bunnell antigen on sheep and bovine RBCs)

Treatment: symptomatic, for uncomplicated mononucleosis

Key Vignette Clues

EBV

- Young adult with fever, lymphadenopathy, splenomegaly
- Downey type II atypical T lymphocytes reach 70% in blood
- Heterophile (monospot) positive

Cytomegalovirus (CMV)

Cytomegalovirus **Reservoir:** humans

Transmission: saliva, sexual, parenteral, in utero

Pathogenesis: CMV infects salivary gland epithelial cells and establishes a persistent infection in fibroblasts, epithelial cells, and macrophages; **latency in mononuclear cells**

Disease

- Cytomegalic inclusion disease: **most common in utero infection in U.S.**; ranges from infected with no obvious defects to severe disease characterized by jaundice, hepatosplenomegaly, thrombocytic purpura ("blueberry muffin baby"), pneumonitis, and CNS damage to death

Key Vignette Clues

CMV

- Heterophile-negative mononucleosis in children and adults
- Neonate with jaundice, hepatosplenomegaly, thrombocytic purpura
- Owl-eye intranuclear inclusion bodies in biopsy

- Mononucleosis (children and adults): heterophile-negative mononucleosis
- Immunocompromised patients
  - Interstitial pneumonitis to severe systemic infection (due to reactivation in transplanted organ or AIDS patient)
  - CMV retinitis common in AIDS patients
  - GI disease common in AIDS patients

Diagnosis

- Owl-eye inclusion ("sight-o-megalo-virus") in biopsy material and urine
- Basophilic intranuclear inclusions
- Serology, DNA detection, virus culture

Treatment: supportive for healthy patients; ganciclovir/foscarnet ± human immunoglobulin for immunocompromised (AIDS and transplant patients) (resistance to ganciclovir through UL97 gene)

Prevention: safe sex; screening of blood and organ donors

Key Vignette Clues

HHV-6

Infant with fever → lacy body rash

HHV-6/7

Reservoir: humans

Transmission: respiratory droplets

Pathogenesis: replicates in peripheral blood mononuclear cells

Disease: roseola (exanthem subitum); fever for 3–5 days followed by lacy body rash

Diagnosis: clinical

Treatment: symptomatic

Key Vignette Clues

HHV-8

AIDS patient with sarcoma

HHV-8 (Kaposi sarcoma-associated herpesvirus KSHV)

Reservoir: humans

Transmission: sexual contact, saliva, vertical, transplantation

Pathogenesis: gene turns on vascular endothelial growth factor (VEGF); plays direct role in development of Kaposi sarcoma; latent in B cells and glandular epithelial cells

Disease: Kaposi sarcoma

Diagnosis: clinical; serology, PCR

Treatment: none

Table II-4-10. Herpesvirus Infections

Virus	Site of Primary Infection	Clinical Presentation of Primary Infection	Site of Latency	Clinical Presentation of Recurrent Infection
HSV-1	Mucosa	Gingivostomatitis, keratoconjunctivitis, pharyngitis	Trigeminal ganglia	Cold sores
HSV-2	Mucosa	Genital herpes, neonatal herpes	Sacral ganglia	Genital herpes
VZV	Mucosa	Chickenpox	Dorsal root ganglia	Shingles (zoster)
EBV	Mucosal epithelial cells, B cells	Mononucleosis (heterophile ⊕)	B cells	Asymptomatic shedding of virus
CMV	Mononuclear cells, epithelial cells	Mononucleosis (heterophile –), cytomegalic inclusion disease	Mononuclear cells	Asymptomatic shedding of virus
HHV-6	Mononuclear cells	Roseola infantum	Mononuclear cells	Asymptomatic shedding of virus
HHV-8	Dermis	Fever, rash	B cells, glandular epithelial cells	Kaposi sarcoma

POXVIRIDAE

Virus Characteristics

- Large dsDNA, enveloped
- Complex morphology
- Replicates in the cytoplasm
- Potential biowarfare agent

Viruses of Medical Importance

- Variola
- Vaccinia (vaccine strain)
- Molluscum contagiosum
- Orf
- Monkeypox

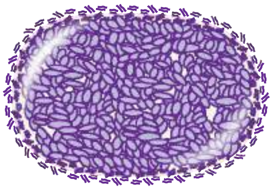


Figure II-4-17. Poxvirus

Key Vignette Clues

Variola/Smallpox

- Virus extinct
- Synchronous rash begins in mouth → face and body
- Guarnieri bodies (intracytoplasmic inclusions)

Variola/Smallpox

Reservoir: humans; Variola has 1 serotype making eradication (1979) possible

Transmission: respiratory route

Pathogenesis

- Via inhalation, the virus enters the upper respiratory tract and disseminates via lymphatics → viremia
- After a secondary viremia, the virus infects all dermal tissues and internal organs
- Classic "pocks"

Disease

- 5–17 day incubation
- Prodrome of flu-like illness for 2–4 days
- Prodrome followed by rash, which begins in mouth and spreads to the face, arms and legs, hands, and feet and can cover entire body within 24 hours
- All vesicles are in same stage of development (synchronous rash)

Diagnosis: clinical; Guarnieri bodies found in infected cells (intracytoplasmic)

Treatment: supportive care

Prevention: live, attenuated vaccine

Key Vignette Clues

Molluscum Contagiosum

- Young adult (wrestling, swim team)
- Umbilicated warts
- Eosinophilic cytoplasmic inclusion bodies

Molluscum contagiosum

Reservoir: humans

Transmission: direct contact (sexual) and fomites

Pathogenesis: replication in dermis

Disease: single or multiple (<20) benign, wart-like tumors; molluscum bodies in central caseous material (eosinophilic cytoplasmic inclusion bodies)

Diagnosis: clinical (warts are umbilicated); eosinophilic cytoplasmic inclusion bodies

Treatment: self-limiting in healthy persons; ritonavir, cidofovir for immunocompromised

RNA VIRUSES: CHARACTERISTICS

All RNA viruses are single-stranded (ss) except Reovirus. ss(−)RNA viruses carry RNA-dependent RNA polymerase. A virion-associated polymerase is also carried by Reovirus, Arenavirus, and Retrovirus (reverse transcriptase).

Most RNA are enveloped; the **only naked ones** are Picornavirus, Calicivirus and Hepevirus, and Reovirus.

Some RNA viruses are segmented, i.e., there are different genes on different pieces of RNA:

- **R**eovirus
- **O**rthomyxovirus
- **B**unyavirus
- **A**renavirus

POSITIVE-STRANDED RNA VIRUSES

Table II-4-11. Positive-Stranded RNA Viruses*

Virus Family	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Multiplies in	Major Viruses
Calicivirus	ss(+)RNA Linear	No polymerase	Naked	Icosahedral	Cytoplasm	Norwalk agent Noro-like virus
Hepevirus	ss(+)RNA Linear	No polymerase	Naked	Icosahedral	Cytoplasm	Hepatitis E
Picornavirus	ss(+)RNA Linear	No polymerase	Naked	Icosahedral	Cytoplasm	Polio** ECHO Enteroviruses Rhino Coxsackie Hepatitis A
Flavivirus	ss(+)RNA Linear	No polymerase	Enveloped	Icosahedral	Cytoplasm	Yellow fever Dengue St. Louis encephalitis Hepatitis C West Nile virus
Togavirus	ss(+)RNA Linear	No polymerase	Enveloped	Icosahedral	Cytoplasm	Rubella WEE, EEE Venezuelan encephalitis
Coronavirus	ss(+)RNA Linear	No polymerase	Enveloped	Helical	Cytoplasm	Coronaviruses SARS-CoV
Retrovirus	Diploid ss (+) RNA Linear	RNA dep. DNA polymerase	Enveloped	Icosahedral or truncated conical	Nucleus	HIV HTLV Sarcoma

*Mnemonic: (+)RNA Viruses: Call Henry Pico and Flo To Come Rightaway
**Mnemonic: Picornaviruses: PEE Co Rn A Viruses
Polio, Entero, Echo, Coxsackie, Rhino, Hep A

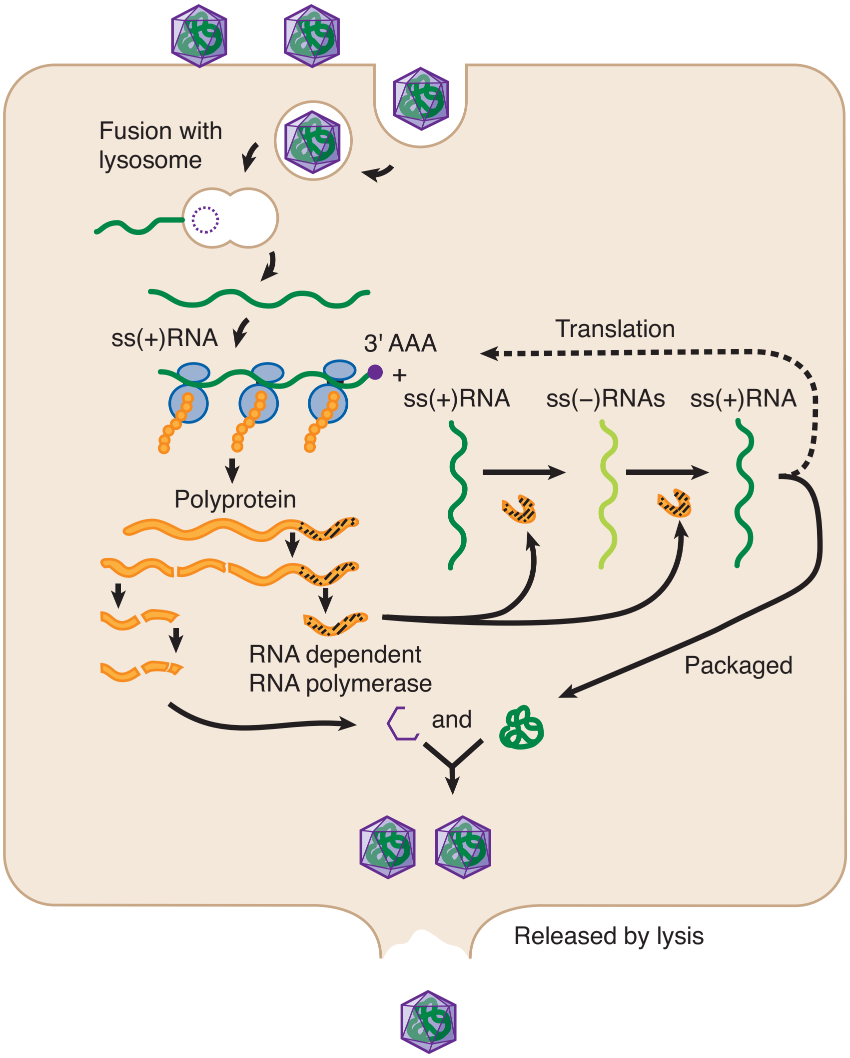


Figure II-4-18. Positive-Sense RNA Virus Life Cycle

CALICIVIRIDAE

Family Characteristics

- Naked, icosahedral
- Positive-sense ssRNA

Viruses of Medical Importance

- Norwalk virus (Norovirus)
- Noro-like virus

Norwalk Virus

Reservoir: human GI tract

Transmission: fecal-oral route, contaminated food and water

Disease: acute gastroenteritis

- Watery; no blood or pus in stools
- Nausea, vomiting, diarrhea
- 60% of all nonbacterial gastroenteritis in U.S.
- Outbreak of viral gastroenteritis in cruise ships attributed to Norovirus

Diagnosis: RIA, ELISA

Treatment: self-limiting; no specific antiviral treatment

Prevention: handwashing

Key Vignette Clues

Norwalk Virus

- School-aged child → adult
- Acute viral gastroenteritis (noninflammatory)

HEPEVIRIDAE

- Naked, icosahedral
- Positive-sense ssRNA

Hepatitis E Virus (previously discussed)

PICORNAVIRIDAE

Family Characteristics

- Small, naked, icosahedral
- Positive-sense ssRNA
- Summer/fall peak incidence
- Resistant to alcohol, detergents (naked capsid)
- Divided into genera:
  - **Enteroviruses**: fecal-oral transmission, do **not** cause diarrhea, peak age <9 years, stable at pH 3
  - **Rhinoviruses**: not stable under acidic conditions, growth at 33 C (91.4 F)
  - **Heparnavirus**

Figure II-4-19. Picornavirus

Viruses of Medical Importance

- Enteroviruses (acid-stable): polio virus; coxsackie virus A; coxsackie virus B; D68; echoviruses
- Rhinoviruses (acid labile)
- Heparnaviruses: HAV

Table II-4-12. Summary of Picornaviridae

Virus	Transmission	Pathogenesis	Diseases	Diagnosis	Treatment/ Prevention
Enteroviruses					
Polio	Fecal-oral	Virus targets anterior horn motor neurons	Asymptomatic to fever of unknown origin; aseptic meningitis; paralytic polio (flaccid asymmetric paralysis, no sensory loss)	Serology (virus absent from CSF)	No specific antiviral/live vaccine (Sabin); killed vaccine (Salk)
		Neural fatigue	Post-Polio Syndrome	Patient with polio decades earlier, progressive muscle atrophy	
Coxsackie A	Fecal-oral	Fecal-oral spread with potential for dissemination to other organs; often asymptomatic with viral shedding	Hand, foot, and mouth (A16); herpangina; aseptic meningitis; acute lymphoglandular pharyngitis; common cold	Virus isolation from throat, stool, or CSF	No specific treatment/ handwashing
Coxsackie B	Fecal-oral	As above	Bornholm disease (devil's grip); aseptic meningitis; severe systemic disease of newborns; **myocarditis**	As above	No specific/ handwashing
D68	Fecal-oral, respiratory	Invade mucosa, lymphatics; potential spread to CNS	Motor-neuron disease; respiratory diseases	Serology/RT-PCR	No specific/IVIG/ handwashing
Echoviruses	Fecal-oral	As above	Fever and rash of unknown origin; aseptic meningitis	As above	No specific/ handwashing
Rhinovirus					
Rhinovirus	Respiratory	Acid labile; grows at 33 C (91.4 F); over 100 serotypes	Common cold; #1 cause, peak summer/ fall	Clinical	No specific/ handwashing
Heparnavirus					
HAV	Fecal-oral	Virus targets hepatocytes; liver function is impaired	Infectious hepatitis	IgM to HAV serology	No specific/ killed vaccine and hyperimmune serum

FLAVIVIRIDAE

Family Characteristics

- Enveloped, icosahedral
- Positive-sense ssRNA
- Arthropod-borne (arboviruses)

Viruses of Medical Importance

- St. Louis encephalitis virus (SLE)
- West Nile encephalitis virus (WNV)
- Dengue virus
- Yellow fever virus (YFV)
- Zika
- Hepatitis C virus (HCV; discussed with the hepatitis viruses)

Table II-4-13. Summary of Flaviviridae

Virus	Transmission	Host(s)	Disease	Diagnosis	Treatment/ Prevention
St. Louis encephalitis virus	Mosquito *(Culex)*	Birds	Encephalitis	Serology, hemagglutination inhibition, ELISA, latex particle agglutination	Symptomatic/ vector control
West Nile encephalitis virus	Mosquito *(Culex)*	Birds (killed by virus)	Encephalitis	As above	Symptomatic/ vector control
Dengue	Mosquito *(Aedes)*	Humans (monkeys)	Break bone fever (rash, muscle and joint pain), reinfection, can result in dengue hemorrhagic shock	As above	Symptomatic/ vector control
Yellow Fever virus	Mosquito *(Aedes)*	Humans (monkeys)	Yellow fever: liver, kidney, heart, and GI (black vomit) damage	As above	Symptomatic/ vector control/ live, attenuated vaccine
Zika	Mosquito *(Aedes)*, vertical, sexual	Vertebrates	Mild → febrile illness of rash and arthralgia Congenital: microcephaly and fetal demise	RT-PCR serology	Symptomatic/ vector control

TOGAVIRIDAE

Family Characteristics

- Enveloped, icosahedral
- Positive-sense ssRNA

Viruses of Medical Importance

- **Alphaviruses (arboviruses)**
  - Eastern equine encephalitis virus (EEE)
  - Western equine encephalitis virus (WEE)
  - Venezuelan equine encephalitis virus (VEE)
  - Chikungunya
- **Rubivirus**
  - Rubella

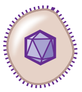


Figure II-4-20. Togavirus

Table II-4-14. Summary of Togaviridae

Virus	Transmission	Host	Disease(s)	Diagnosis	Prevention
Eastern equine encephalitis virus (EEE), Venezuelan equine encephalitis virus (VEE), Western equine encephalitis virus (WEE)	Mosquito	Birds, horses	Encephalitis	Cytopathology, immunofluorescence, RT-PCR, serology	Killed vaccines for EEE and WEE
Chikungunya	Mosquito (*Aedes*)	Primates, rodents, birds	Febrile polyarthralgia, arthritis		
Rubella	Respiratory	humans	German measles (erythematous rash begins on face, progresses to torso)	Serology	Live, attenuated vaccine
	Vertical	humans	Congenital rubella syndrome*		

*Congenital rubella syndrome: patent ductus arteriosus, pulmonary stenosis, cataracts, microcephaly, deafness (effects are more serious if maternal infection is acquired during first 16 weeks' gestation)

CORONAVIRIDAE

Family Characteristics

- Enveloped, helical
- Positive-sense ssRNA
- Hemagglutinin molecules make up peplomers on virus surface, which give shape like sun with corona

Viruses of Medical Importance

- **Coronavirus**
- **Severe acute respiratory syndrome coronavirus** (SARS-CoV)

Coronavirus

- Second most common cause of the common cold
- Winter/spring peak incidence

SARS-CoV

Reservoir: birds and small mammals (civet cats)

Transmission: respiratory droplets; virus also found in urine, sweat, and feces; original case is thought to have jumped from animal to human

Disease: severe acute respiratory syndrome (SARS)

- Atypical pneumonia
- Clinical case definition includes fever of >38.0 C (100.4 F), flu-like illness, dry cough, dyspnea, and progressive hypoxia
- Chest x-ray may show patchy distribution of focal interstitial infiltrates

Diagnosis

- Includes clinical presentation and prior history of travel to endemic area or an association with someone who recently traveled to endemic area
- Lab tests: detection of antibodies to SARS-CoV, RT-PCR, and isolation of the virus in culture

Treatment: supportive; ribavirin and interferon are promising

MERS-CoV (Middle Eastern Respiratory Syndrome)

Reservoir: bats and camels

Disease and transmission: similar to SARS

Key Vignette Clues

SARS-CoV

- Patient with acute respiratory distress
- Travel to Far East or Toronto
- Winter/spring peak incidence

RETROVIRIDAE

Family Characteristics

- Positive-sense ssRNA
- Virion-associated reverse transcriptase
- Enveloped

Viruses of Medical Importance

- **Oncovirus group**
  - **Human T-cell leukemia/lymphotropic (HTLV)**
    - Adult T-cell leukemia, HTLV-1 associated myelopathy (HAM)
    - Causes proliferation of T cells and transformation
    - Tax gene required for transformation
    - Clover leaf nucleus
    - C-type particle (most oncoviruses, centrally located electron-dense nucleocapsid)
    - Japan, Caribbean, South America
- **Lentivirus group**: **human immunodeficiency virus (HIV);** acquired immunodeficiency syndrome

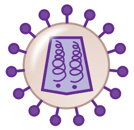


Figure II-4-21. Retrovirus

Key Vignette Clues

HIV

- Homosexual men, IV drug user, sexually active adult
- Decreasing CD4 cell count
- Opportunistic infections
- Fatigue, weight loss, lymphadenopathy, low-grade fever

Human Immunodeficiency Virus (HIV)

Distinguishing Characteristics

- HIV virion contains:
  - Enveloped, truncated, conical capsid (type D retrovirus)
  - Two copies of ss(+)RNA
  - RNA-dependent DNA polymerase (reverse transcriptase)
  - Integrase
  - Protease

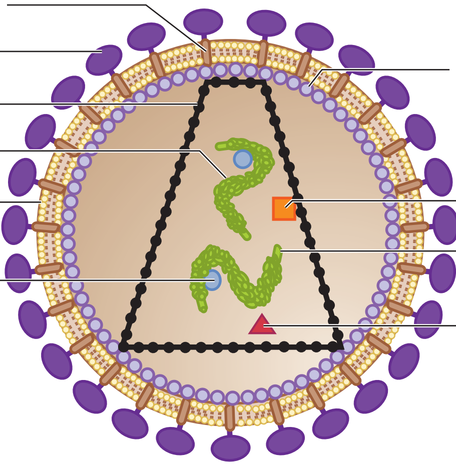


Figure II-4-22. Structure and Genes of HIV

Table II-4-15. Important HIV Genes and Their Functions

Gene	Product(s)	Function
Structural Genes		
Gag	Group-specific antigens	Structural proteins
	p24	Capsid protein
	p7p9	Core nucleocapsid proteins
	p17	Matrix protein
Pol	Reverse transcriptase	Produces dsDNA provirus (extremely error-prone, causes genetic drift of envelope glycoprotein)
	Integrase	Viral DNA integration into host cell DNA
	Protease	Cleaves viral polyprotein
Env	gp120	Surface protein that binds to CD4 and coreceptors CCR5 (macrophages) and CXCR4 (T cells); tropism; genetic drift
	gp41	Transmembrane protein for viral fusion to host cell

Gene	Product(s)	Function
Regulatory Genes		
LTR **(U3, U5)**	DNA, long terminal repeats	Integration and viral gene expression
Tat	Transactivator	Transactivator of transcription (upregulation); spliced gene
Rev	Regulatory protein	Upregulates transport of unspliced and spliced transcripts to the cell cytoplasm; a spliced gene
Nef	**Regulatory protein**	**Decreases CD4 and MHC I expression on host cells; manipulates T-cell activation pathways; required for progression to AIDS**

The clinical manifestations of mutations of regulatory genes are as follows:

Gene	From	Mutation	Outcome
Nef	HIV	Loss of function	Slow rate of progression to AIDS or no progression to AIDS (non-progressor)
Tat/*rev*	HIV	Loss of function	Virus unable to replicate
Vif	HIV	Loss of function	Slow rate of progression (non-progressor)
CCR5	HIV	Host cell macrophages/DCs	Homozygous: patient immune to HIV Heterozygous: slow progression to AIDS

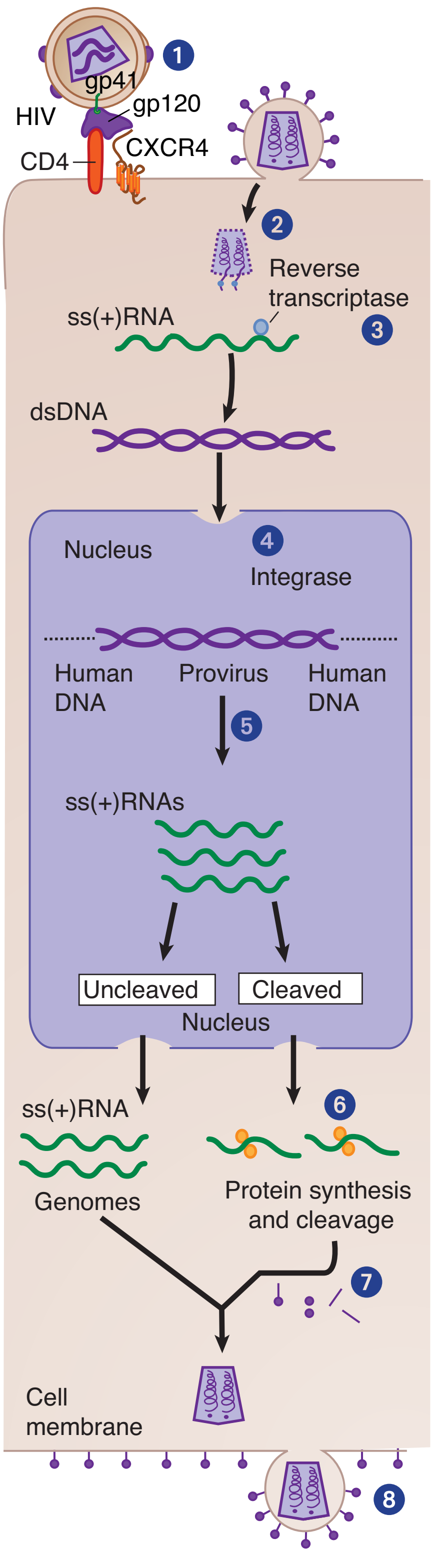


Figure II-4-23. Retrovirus Life Cycle: HIV

1. Surface gp120 of HIV binds to CD4 of T-helper cells, macrophages, microglia, and coreceptors (*CCR5* and *CXCR4*) found on macrophages and Th cells, respectively.
2. HIV is taken into the cell, losing the envelope; the RNA is uncoated.
3. The RNA is copied using the virion-associated reverse transcriptase; ultimately dsDNA with long terminal repeats is made.
4. The DNA and integrase migrate to nucleus, and the DNA is integrated into host DNA forming the **provirus**. The provirus remains in the host DNA.

   The rate of viral replication is regulated by the activity of the regulatory proteins (*tat/rev, nef,* etc). ***Tat*** upregulates transcription. ***Rev*** regulates transport of RNA to cytoplasm.

   Co-infections (e.g., mycobacterial) stimulate the HIV-infected cells to produce more virus.
5. Transcription produces ss(+)RNA, some cleaved and some remain intact.
   - Cleaved RNA will be used as mRNA.
   - Uncleaved RNA is used as genomic RNA.
6. Translation produces the proteins, some of which are polyproteins that are cleaved by the HIV protease.
7. Assembly
8. Maturation/release of virus

Reservoir: human Th cells and macrophages

Transmission: sexual contact, bloodborne (transfusions, dirty needles), vertical

Disease: acquired immunodeficiency syndrome (AIDS)

- Asymptomatic infection → persistent, generalized lymphadenopathy → symptomatic → AIDS-defining conditions
- Homozygous *CCR5* mutation → immune
- Heterozygous *CCR5* mutation → slow course
- Course of the illness follows decline in CD4+ T cells
- Long-term survivors may result when virus lacks functional *nef* protein

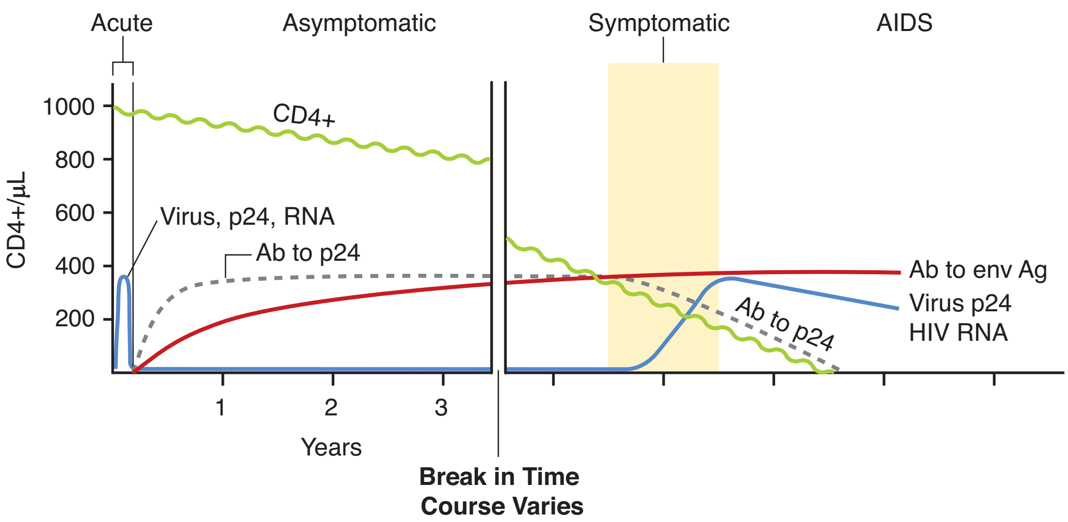


Figure II-4-24. Clinical Stages of HIV Infection

Stage is based primarily on CD4+ T-lymphocyte count; the CD4+ T-lymphocyte count takes precedence over CD4 T-lymphocyte percentage, and the percentage is considered only if count is missing.

Table II-4-16. HIV Infection Stage* based on age-specific CD4+ T-Lymphocyte Count or CD4+ T-Lymphocyte Percentage of Total Lymphocytes

Stage	Age on date of CD4+ T-lymphocyte test		
	Age <1 yr	Age 1–5 yrs	Age ≥6 yrs
1	≥1,500 cells/µL, ≥34%	≥1,000 cells/µL, ≥30%	≥500 cells/µL, ≥26%
2	750–1,499 cells/µL, 26–33%	500–999 cells/µL, 22–29%	200–499 cells/µL, 14-25%
3	<750 cells/µL, <26%	<500 cells/µL, <22%	<200 cells/µL, <14%

There are 3 situations in which stage is not based on these criteria: 1) if criteria for stage 0 are met, the stage is 0 regardless of criteria for other stages (CD4 T-lymphocyte test results and opportunistic illness diagnoses); 2) if criteria for stage 0 are not met and a stage-3-defining opportunistic illness has been diagnosed, then stage is 3 regardless of CD4 T-lymphocyte test results; or 3) if criteria for stage 0 are not met and information on the above criteria for other stages is missing, then stage is unknown.

Conditions of Early Symptomatic Period

- Bacillary angiomatosis (disseminated bartonellosis)
- Candidiasis (oral or persistent vulvovaginal)
- Cervical dysplasia or carcinoma in situ
- Constitutional symptoms (fever 38.5 C [101.3 F] or diarrhea lasting >1 month)
- Hairy leukoplakia
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease (especially with abscess)
- Peripheral neuropathy

Conditions Associated with AIDS

- Encephalopathy, HIV-related
- Pneumonia, recurrent (leading cause of death)
- Fungal infections
- Candidiasis of esophagus, bronchi, trachea, or lungs
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Histoplasmosis, disseminated or extrapulmonary

- *Pneumocystis jirovecii* pneumonia
- Malignancies: invasive cervical carcinoma; Kaposi sarcoma; Burkitt, immunoblastic, or primary CNS lymphoma
- Viral infections: cytomegalovirus retinitis (with loss of vision) or disease (other than liver, spleen, or nodes); herpes simplex: chronic ulcer(s) (>1 month) or bronchitis, pneumonitis, or esophagitis; progressive multifocal leukoencephalopathy; wasting syndrome due to HIV (TNF-α)
- Parasitic infections: cryptosporidiosis, chronic intestinal (>1 month); isosporiasis, chronic intestinal (>1 month); toxoplasmosis of brain
- Bacterial infections
  - *Mycobacterium tuberculosis,* any site (pulmonary or extrapulmonary)
  - *Mycobacterium avium* complex or *M. kansasii* or other species or unidentified species, disseminated or extrapulmonary
  - *Salmonella* septicemia, recurrent

Table II-4-17. Recommended Prophylactic Regimens during HIV Infection

Disease Agent	Begin Prophylaxis
Pneumocystis jirovecii	<200 CD4
Toxoplasma gondii	<100 CD4
Histoplasma capsulatum	<100 CD4 (in endemic area)
Mycobacterium avium intracellulare	<50 CD4
Cytomegalovirus	<50 CD4
Cryptococcus neoformans	<50 CD4

Table II-4-18. Laboratory Analysis for HIV

Purpose	Test
Initial screening	Serologic: ELISA (HIV1 and HIV2 antibodies, p24 antigen)
Confirmation	Nucleic acid test (NAT)
Detection of virus in blood (evaluate **viral load**)	RT-PCR*
Detect HIV infection in newborns of HIV+ mother **(provirus)**	PCR*
Early marker of infection	p24 antigen
Evaluate progression of disease	CD4:CD8 T-cell ratio

*RT-PCR tests for circulating viral RNA and is used to monitor the efficacy of treatment. PCR detects integrated virus (provirus). Viral load has been demonstrated to be the best prognostic indicator during infection.

Table II-4-19. Treatment

Mechanism	Name	Resistance
RT inhibitors Nucleoside or non-nucleoside analogs	End in "ine"	Common, leads to cross-resistance
Protease inhibitors	End in "inavir"	Common via protease mutations, leads to cross-resistance
HAART (highly active anti-retroviral therapy)	2 nucleoside analogs and 1 protease inhibitor	Increasing
Fusion inhibitors	Fuzeon, enfuvirtide	Not yet
CCR5 co-receptor antagonist	Maraviroc	Not yet
Integrase inhibitor	Raltegravir	Not yet

Prevention: safe sex/education; blood/organ screening; infection control; vaccine development (currently none available)

NEGATIVE-STRANDED RNA VIRUSES

Table II-4-20. Negative-Stranded RNA Viruses

Virus	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Multiplies in	Major Viruses
Paramyxovirus	ss(–)RNA Linear Non-segmented	Yes	Yes	Helical	Cytoplasm	Mumps Measles Respiratory syncytial Parainfluenza
Rhabdovirus	ss(–)RNA Linear Non-segmented	Yes	Yes	Helical, bullet-shaped	Cytoplasm	Rabies Vesicular stomatitis
Filovirus	ss(–)RNA Linear Non-segmented	Yes	Yes	Helical	Cytoplasm	Marburg Ebola
Orthomyxovirus	ss(–)RNA Linear 8 segmented	Yes	Yes	Helical	Cytoplasm & nucleus	Influenza
Bunyavirus	ss(–)RNA Pseudocircular, 3 segments, 1 is ambisense	Yes	Yes	Helical	Cytoplasm	California encephalitis La Crosse encephalitis Hantavirus
Arenavirus	ss(–)RNA Circular 2 segments 1 (–)sense 1 ambisense	Yes	Yes	Helical	Cytoplasm	Lymphocytic choriomeningitis Lassa fever

Mnemonic for ss(–)RNA viruses: Pain Results From Our Bunions Always. Gives them in order of size. Remember that these are the negative ones because pain is a negative thing. Another one: Bring a polymerase or fail replication.

Note that all are enveloped, all have virion-associated polymerase, and all have helical nucleocapsids. The oddballs are the last three:

- The orthomyxoviruses are linear (ortho) but with 8 (ortho/octo) segments, which is one reason they can genetically "mix" it up. The orthomyxoviruses are also odd in that they replicate in both the nucleus and cytoplasm.
- The bunyaviruses are somewhat contortionists (circular): 3 Californian contortionists
- The arenaviruses have one negative sense and one ambisense strand of RNA.

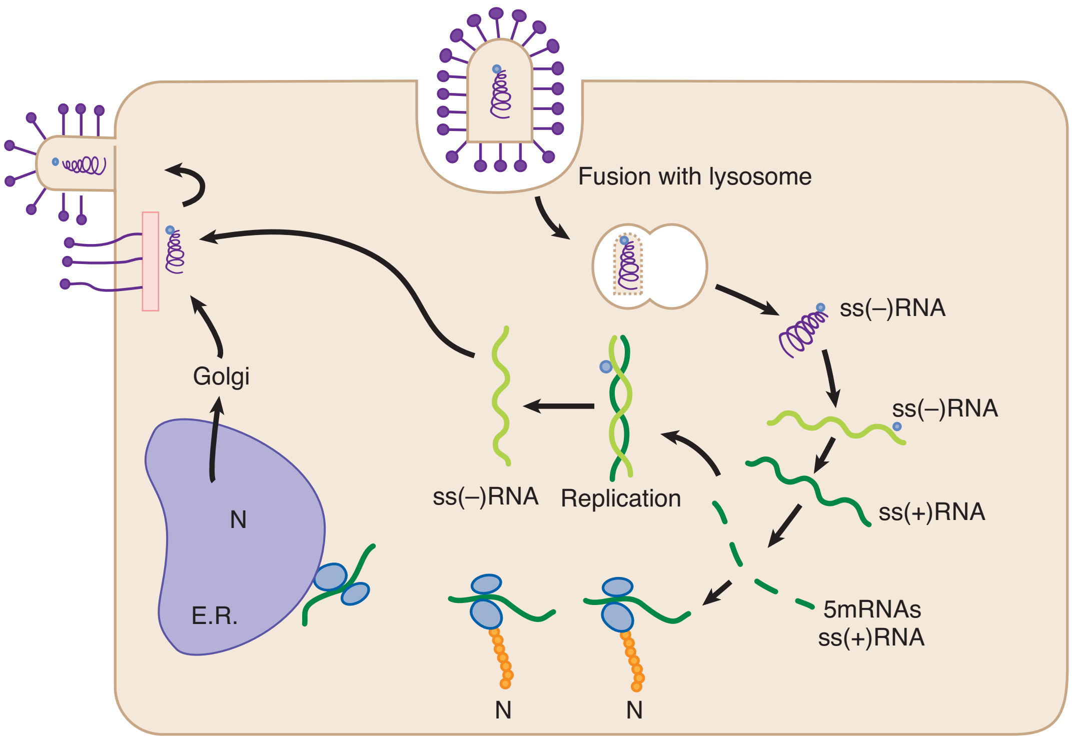


Figure II-4-25. Negative-Sense RNA Virus Life Cycle

PARAMYXOVIRIDAE

Family Characteristics

- Enveloped, helical nucleocapsid
- Negative-sense ssRNA

Viruses of Medical Importance

- Measles
- Mumps
- Parainfluenza
- Respiratory syncytial virus (RSV)
- Human metapneumovirus (human MNV)

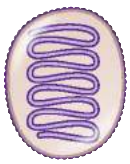


Figure II-4-26. Paramyxovirus

Measles Virus

Distinguishing Characteristics: single serotype; H-glycoprotein and fusion protein; no neuraminidase

Reservoir: human respiratory tract

Transmission: respiratory route

Pathogenesis: ability to cause cell: cell fusion → giant cells; virus can escape immune detection

Disease

- Measles: presentation generally 3 Cs (cough, coryza, and conjunctivitis) with photophobia; Koplik spots → maculopapular rash from ears down → giant cell pneumonia (Warthin-Finkeldey cells)
- Subacute sclerosing panencephalitis: rare late complication (mean time 7–10 years); mutant measles virus persists in brain, acts as slow virus; chronic CNS degeneration

Diagnosis: serology

Treatment: supportive, ribavirin (experimental)

Prevention: live, attenuated vaccine, MMR

Mumps Virus

Distinguishing Characteristics: negative-sense ssRNA; helical; enveloped; single HN glycoprotein, also F protein; single serotype

Reservoir: human respiratory tract

Transmission: person to person via respiratory droplets

Pathogenesis: lytic infection of epithelial cells of upper respiratory tract and parotid glands → spread throughout body

Disease: mumps

- Asymptomatic to bilateral parotitis with fever, headache, and malaise
- Complications include pancreatitis, orchitis (leads to sterility in males), and meningoencephalitis

Diagnosis: clinical; serology; ELISA, IFA, hemagglutination inhibition

Treatment: supportive

Prevention: live, attenuated vaccine, MMR

Table II-4-21. Additional Paramyxoviruses

Virus	Transmission	Disease(s)	Diagnosis	Treatment/ Prevention
Parainfluenza	Respiratory	Older children and adults: subglottal swelling; hoarse, barking cough Infants: colds, bronchiolitis, pneumonia, **croup**	RT-PCR	Supportive/none
RSV	Respiratory	Adults: colds; infants/**preemies**: bronchiolitis and necrosis of bronchioles, atypical pneumonia (low fever, tachypnea, tachycardia, expiratory wheeze)	Indirect fluorescent antibody, enzyme-linked immunosorbent assay, RT-PCR	Ribavirin and anti-RSV Abs/ none Palivizumab blocks fusion protein
Human Metapneumovirus	Respiratory	Common cold (15% in kids), bronchiolitis, pneumonia	RT-PCR	Supportive/none

Definition of abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction

RHABDOVIRIDAE

Family Characteristics

- Negative-sense ssRNA
- Bullet shaped
- Enveloped, helical

Viruses of Medical Importance

- Rabies

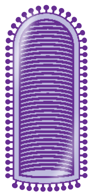


Figure II-4-27. Rhabdovirus

Key Vignette Clues

Rabies

- Patient bitten by bat or dog
- Influenza-like prodrome: hydrophobia, hallucination, coma, death

Rabies Virus

Reservoir: In U.S., most cases are sylvatic (bats, raccoons, foxes, skunks); worldwide, mostly dogs

Transmission: bite or contact with rabid animal

Pathogenesis: After contact, virus binds to peripheral nerves by binding to nicotinic acetylcholine receptor **or** indirectly into the muscle at site of inoculation; virus travels by **retrograde axoplasmic** transport to dorsal root ganglia and spinal cord; once virus gains access to spinal cord, brain becomes rapidly infected

Disease: rabies

- Nonspecific flu-like illness followed by neurologic symptoms of **hydrophobia**, seizures, disorientation**, hallucination**, coma, and death
- With rare exception, rabies fatal unless treated by immunoprophylaxis

Diagnosis: clinical; Negri bodies, intracytoplasmic inclusion bodies (brain biopsy); DFA (impression smears of corneal epithelial cells), PCR (usually too late)

Treatment: none if symptoms are evident; if suspect: postexposure prophylaxis 1 dose of human rabies immunoglobulin (hRIG); 4 doses of rabies vaccine (day of and then days 3, 7, 14); killed virus vaccine

Prevention: vaccine for high-risk individuals; vaccination program for domestic animals (U.S.)

FILOVIRIDAE

Family Characteristics

- Negative-sense ssRNA
- Enveloped, helical
- Filamentous

Viruses of Medical Importance

- Ebola virus : Zaire species most virulent (2014–2015 outbreak in West Africa), 70% mortality
- Marburg virus

Reservoir

- Ebola: bats suspected
- Marburg: bats confirmed

Transmission (Ebola)

- Contact with body fluids, meat of infected animals
- Direct contact with infected patients, body fluids, blood, skin

Pathogenesis (Ebola)

- Virus infects many cell types (endothelial, epithelial, fibroblasts, etc); macrophages, and dendrite cells first cells likely to be infected
- Virus delivered to regional lymph nodes, then to blood stream
- Systemic inflammatory response induces cytokine storm, which results in tissue damage including vascular leak, SI dysfunction, and eventual multi-organ failure

Ebola

Disease

- Incubation period ~6–12 days
- Non-specific flu-like illness (fever, chills, general malaise)
- Fatigue, headaches, vomiting and diarrhea
- Massive hemorrhage not common, although bleeding via bloody stool, petechiae, or mucosal bleeding can be
- GI manifestations important in severe illness, with vomiting and diarrhea leading to fluid loss
- Rash and meningoencephalitis may occur

Diagnosis

- Determine likelihood of exposure
- RT-PCR (confirmatory); rapid antigen test (screen) must also be confirmed with RT-PCR

Treatment

- Symptomatic
- Non-approved anti-virals (clinical trials): favipiravir, brincidofovir
- Ebola-specific treatments in development include monoclonal antibodies targeting surface GP B and anti-RNA

Prevention

- Infection control precautions: quarantine, travel restrictions
- Vaccines in development

ORTHOMYXOVIRIDAE

Family Characteristics

- Negative-sense ssRNA
- Enveloped
- Segmented (8 segments)
- Helical

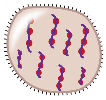


Figure II-4-28. Orthomyxovirus

Viruses of Medical Importance

- Influenza A
- Influenza B

Key Vignette Clues

Influenza

Patient with headache, malaise, fever, myalgia, cough

Influenza Virus

Distinguishing Features

- Envelope contains two glycoproteins, H and N
- Used to serotype virus

Reservoir

- Influenza A (birds, pigs, humans)
- Influenza B (humans only)

Transmission

- Direct contact
- Respiratory
- 1997 H5N1 strain jumped directly from birds to humans
- 2009 H1N1 strain—quadruple reassortment virus (North American swine, avian, human; Asian and European swine)

Pathogenesis

- Antigenic drift
  - Influenza A and B
  - Slight changes in antigenicity due to mutations in H and/or N
  - Causes epidemics

- Antigenic shift
  - *Influenza A only*
  - Rare genetic reassortment
  - Coinfection of cells with two different strains of influenza A (H5N1 and H3N2); reassortment of segments of genome
  - Production of a new agent to which population has no immunity
  - Responsible for pandemics

Disease: influenza

- Headache and malaise
- Fever, chills, myalgias, anorexia
- Bronchiolitis, croup, otitis media, vomiting (younger children)
- Pneumonia/secondary bacterial infections
- Can lead to Reye syndrome or Guillain-Barré syndrome

Diagnosis

- Rapid tests (serology)
- Clinical symptoms plus season

Treatment

- Amantadine/rimantadine (current isolates are commonly resistant)
  - Inhibit viral uncoating
  - Administer orally
- Zanamivir/oseltamivir
  - Neuraminidase inhibitors
  - Zanamivir is inhaled
  - Oseltamivir is given orally

Prevention

- Killed vaccine
  - Two strains of influenza A (H3N2, H1N1, for example) and one strain of influenza B are incorporated into the vaccine
- Live, attenuated vaccine
  - Intranasal administration
  - Similar composition
  - No longer recommended

Key Vignette Clues

Hantavirus

- Patient with acute respiratory distress
- Four-corners region
- Exposure to rodent excrement
- Spring/early summer incidence

BUNYAVIRIDAE

Family Characteristics

- Negative-sense ssRNA
- Enveloped viruses
- Three segments, one ambisense
- Mostly arboviruses, except Hantavirus

Viruses of Medical Importance

- California encephalitis
- LaCrosse encephalitis
- Hantavirus (sin nombre)

Table II-4-22. Bunyaviridae

Virus	Transmission	Disease	Diagnosis
California and LaCrosse encephalitis	Mosquito	Viral encephalitis	Serology
Hantavirus (sin nombre)	Rodent excrement, four-corners region, rainy season	Hantavirus pulmonary syndrome (cough, myalgia, dyspnea, tachycardia, pulmonary edema and effusion, and hypotension [mortality 50%])	RT-PCR

ARENAVIRIDAE

Family Characteristics

- Negative-sense ssRNA
- Pleomorphic, enveloped
- Virions have a sandy appearance (ribosomes in virion)
- Two segments, one ambisense

Viruses of Medical Importance

- Lymphocytic choriomeningitis virus (LCMV)
- Lassa fever virus

Table II-4-23. Arenaviridae

Virus	Transmission	Disease	Diagnosis	Treatment
Lymphocytic choriomen-ingitis virus	Mice and pet hamsters (U.S.)	Influenza-like with meningeal signs	Serology, level 3 isolation	Supportive, ribavirin
Lassa fever	Rodents, human-to-human (West Africa)	Hemorrhagic fever with 50% fatality rate	Serology, level 4 isolation	Supportive, ribavirin

DOUBLE-STRANDED RNA VIRUSES

REOVIRIDAE

Table II-4-24. Double-Stranded RNA Viruses

	RNA Structure	Polymerase	Envelope	Shape	Major Viruses
Reovirus	Linear dsRNA 10-11 segments	Yes	Naked	Icosa-hedral Double shelled	Reovirus Rotavirus Colorado Tick Fever Virus

Figure II-4-29. Reovirus

Table II-4-25. Reoviridae

Virus	Transmission	Disease	Diagnosis	Treatment/Prevention
Reovirus	Fecal-oral, respiratory	Common cold, gastroenteritis	Serology	Self-limiting/none
Rotavirus	Fecal-oral	Gastroenteritis, no blood or pus	Enzyme-linked immunosorbent assay (stool)	Live, attenuated vaccine, oral

PRION DISEASES

Table II-4-26. Prion Diseases

Disease	Infectious agent	Host	Comments
Kuru	Prion	Human	Subacute spongiform encephalopathy (SSE); Fore Tribe, New Guinea; cannibalism
Creutzfeldt-Jakob disease (and variant)	Prion	Human	SSE PrPSc: Humalog of normal protein PrPC found in normal brain and tissue Genetic predisposition; ingestion of infected cow brains
Gerstmann-Sträussler-Scheinker	Prion	Human	SSE
Fatal familial insomnia	Prion	Human	SSE
Scrapie	Prion	Sheep	SSE: scraping their wool off on fences

Table II-4-27. Slow Conventional Viruses (Viruses)

Disease	Infectious agent	Host	Comments
Measles SSPE	Virus	Human having had measles	Subacute sclerosing panencephalitis
AIDS dementia	HIV	Human	Dementia
PML	JC virus	Human	Progressive multifocal leukoencephalopathy

Medically Relevant Fungi

5

Learning Objectives

- ❏ Demonstrate understanding of mycology and fungal morphology
- ❏ Differentiate between nonsystemic fungal infections and deep fungal infections

MYCOLOGY

Mycology is the **study of fungi (molds, yeasts, and mushrooms).** All fungi have the following characteristics:

- **Eukaryotic** (e.g., true nucleus, 80S ribosomes, mitochondria, as are humans)
- **Complex carbohydrate cell walls**: chitin, glucan, and mannan
- **Ergosterol** is major membrane sterol
  - Imidazole antifungals inhibit synthesis of ergosterol
  - Polyene antifungals bind more tightly to ergosterol than to cholesterol

FUNGAL MORPHOLOGY

Hyphae are filamentous cellular units of molds and mushrooms.

- **Nonseptate hyphae** have no cross walls, broad hyphae with **irregular width**, and **broad angle of branching**.
- **Septate hyphae** have **cross walls** and fairly regular width (tube-like).

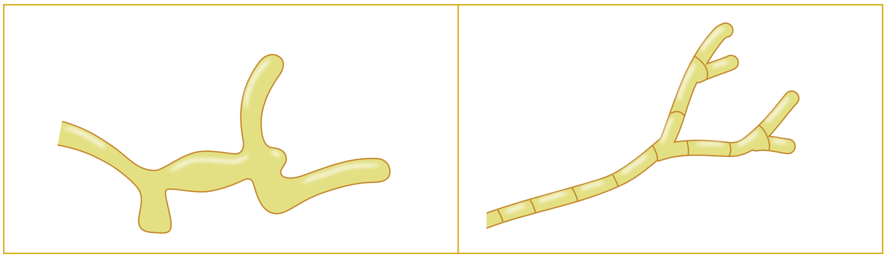


Figure II-5-1. Hyphae Types

The **mycelium** is the mat of hyphae. **Yeasts** are single-celled (round to oval) fungi.

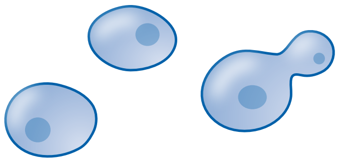


Figure II-5-2. Yeasts

Fungi able to convert from hyphal to yeast (or yeast-like) forms are called **dimorphic fungi**. They are **thermally dimorphic:** mold in the **"cold"** (25.0 C [77.0 F]), yeast in the beast (37.0 C [98.6 F]).

Key dimorphic fungi include *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Sporothrix*.

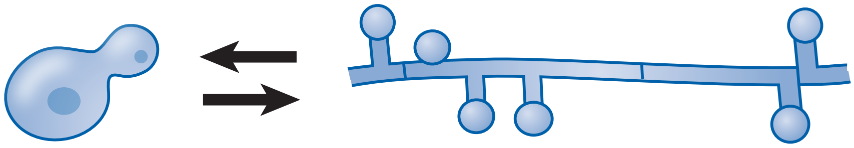


Figure II-5-3. Dimorphic Fungi

Pseudohyphae (*Candida albicans*) are hyphae with constrictions at each septum.

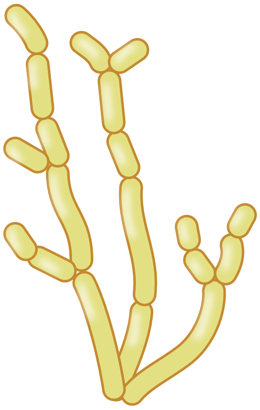


Figure II-5-4. *Candida* Pseudohyphae

Spore types include:

- Conidia: **asexual spores**; formed off of hyphae; common; airborne
- Blastoconidia: "buds" on yeasts (asexual budding daughter yeast cells)
- Arthroconidia: asexual spores formed by a **"joint"**
- Spherules and endospores *(Coccidioides)*: spores **inside spherules** in tissue

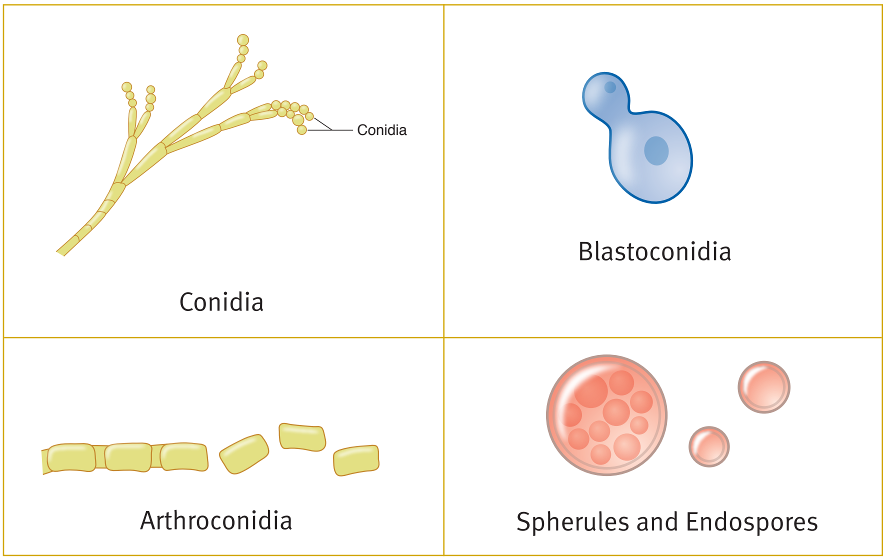


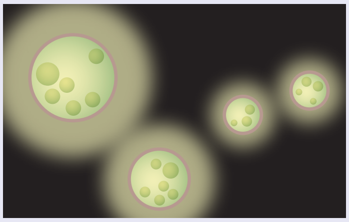
Figure II-5-5. Spore Types

Diagnosis

Diagnosis begins with microscopic methods and specialized stains. Diagnosis then continues with the following:

- **Culture** (may take several weeks): special fungal media include inhibitory mold agar as modification of Sabouraud with antibiotics:
  - **Sabouraud agar** and blood agar (both with antibiotics)
  - Culture identifies fungal **morphology** and PCR with nucleic acid probes
- **Serology** includes antibody screen and complement fixation and looks for patient antibody
- **Fungal antigen detection** (CSF, serum): **cryptococcal capsular polysaccharide detection by latex particle agglutination** (LPA) or counter immunoelectrophoresis
- **Skin test** is most useful for **epidemiology** or **demonstration of anergy** to an agent you know patient is infected with (grave prognosis); otherwise, like tuberculosis, skin test **only indicates exposure** to agent

Table II-5-1. Microscopic Methods/Special Fungal Stains

Preparation	Fungal Color	Notes
KOH wet mount (KOH degrades human tissues leaving hyphae and yeasts visible)	Colorless (hyaline) refractive green or light olive to brown (dematiaceous) fungal elements	Heat gently; let sit 10 min; dissolves human cells
PAS	Hot pink	
Silver stain	Gray to black	*Pneumocystis*
Calcofluor white (can be done on wet mounts)	Bright blue-white on black	Scrapings or sections; fluorescent microscope needed
India ink wet mount of CSF sediment	Colorless cells with halos (capsule) on a black particulate background (*Cryptococcus neoformans*)	Only "rules in"; insensitive; misses 50%  *Cryptococcus neoformans*

NONSYSTEMIC FUNGAL INFECTIONS

Superficial Infections (Keratinized Tissues)

Malassezia furfur

Normal skin flora (lipophilic yeast)

Diseases include:

- **Pityriasis or tinea versicolor**
  - Superficial infection of keratinized cells
  - Moist, warm climates predispose
  - **Hypopigmented spots on chest/back** (blotchy suntan)
  - KOH mount of skin scales: spaghetti and meatballs (bacon and eggs); **yeast clusters and short, curved septate hyphae**
  - Coppery-orange fluorescence under Wood lamp (UV)
  - Treatment is topical selenium sulfide or topical azole antifungal
- **Fungemia in premature infants** on intravenous lipid supplements

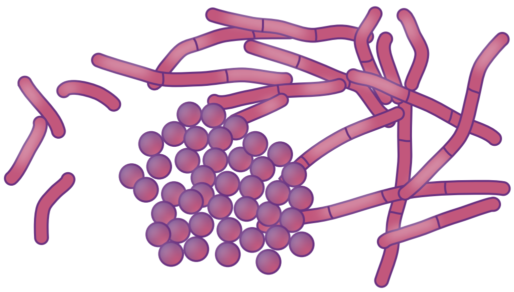


Figure II-5-6. *Malassezia furfur*

Cutaneous Fungal Infections (without Systemic Disease)

Yeast skin infections

- Commonly **cutaneous or mucocutaneous candidiasis**
- May disseminate in compromised patients
- Discussed with opportunistic fungi

Dermatophytes (group of fungi)

- **Filamentous** fungi (**monomorphic**)
- **Infect only skin and hair and/or nails** (do not disseminate)
- Three genera:
  - ***Trichophyton***: infects skin, hair, and nails
  - ***Microsporum***: infects hair and skin
  - ***Epidermophyton***: infects nails and skin

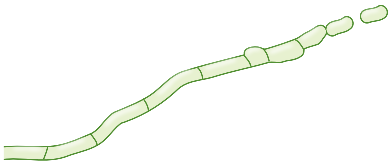


Figure II-5-7. Dermatophyte

Diseases include **dermatophytic infections** (tineas, also called ringworm). **Itching** is the most common symptom of all tineas.

- **Tinea capitis**: ringworm of the scalp (most serious form is **favus** (tinea favosa), which is very contagious and causes **permanent hair loss**)
- **Tinea barbae**: ringworm of the bearded region
- **Tinea corporis**: dermatophytic infection of the glabrous skin
- **Tinea cruris**: jock itch
- **Tinea pedis**: athlete's foot
- **Tinea unguium**: ringworm of the nails

If symptoms include **high inflammation**, it is generally from **animals** (zoophilic). If symptoms include **little inflammation**, it is generally from **humans**.

Diagnosis is made with Microsporum (fluoresces a bright yellow-green [Wood lamp]) and **KOH mount** of nail or skin scrapings (shows **arthroconidia** and **hyphae**).

Treatment is topical imidazole or tolnaftate (use oral imidazole or griseofulvin where hairs are infected or skin contact hurts). Keep areas **dry**.

ID reaction

(Dermatophyt<u>id</u>) = allergic response to circulating fungal antigens

Subcutaneous Mycoses

Sporothrix schenckii

Dimorphic fungus

Environmental form: on **plant material**, worldwide as **hyphae with rosettes and sleeves of conidia**

- **Traumatic implantation** (rose or plum tree thorns, wire/sphagnum moss)

Tissue form: cigar-shaped yeast in tissue

Diseases include:

- **Sporotrichosis (rose gardener disease): subcutaneous or lymphocutaneous lesions**; treatment: itraconazole or **potassium iodide in milk**
- **Pulmonary** (acute or chronic) **sporotrichosis**; urban alcoholics, particularly homeless (**alcoholic rose-garden-sleeper disease**)

Treatment is itraconazole for bone/joint infection and amphotericin B for severe/systemic infection.

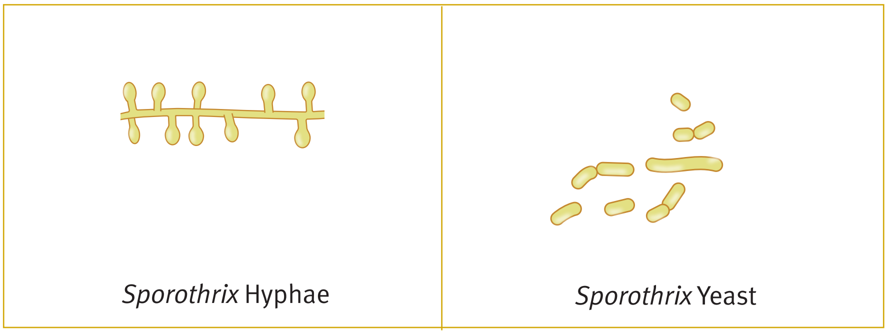


Figure II-5-8. *Sporothrix*

SYSTEMIC FUNGAL INFECTIONS

There are 4 important **classical pathogens** in the United States. All of them cause **acute pulmonary** (asymptomatic or self-resolving in 95% of cases), **chronic pulmonary**, or **disseminated infection**.

- *Histoplasma*
- *Coccidioides*
- *Blastomyces*
- *Paracoccidioides*

Diagnosis is made with sputum cytology (calcofluor white helpful); **sputum culture** on blood agar; and **special fungal media** (inhibitory mold agar, Sabouraud). Peripheral blood culture is useful for *Histoplasma* since it circulates in reticuloendothelial system (RES) cells.

Histoplasma Capsulatum

Dimorphic fungus

Environmental form: hyphae with **microconidia** and **tuberculate macroconidia**

- Endemic region: **Eastern Great Lakes, Ohio, Mississippi, and Missouri River beds**
- **Found in soil (dust) enriched with bird or bat feces**
- Spelunking **(cave exploring),** cleaning **chicken coops,** or bulldozing starling roosts

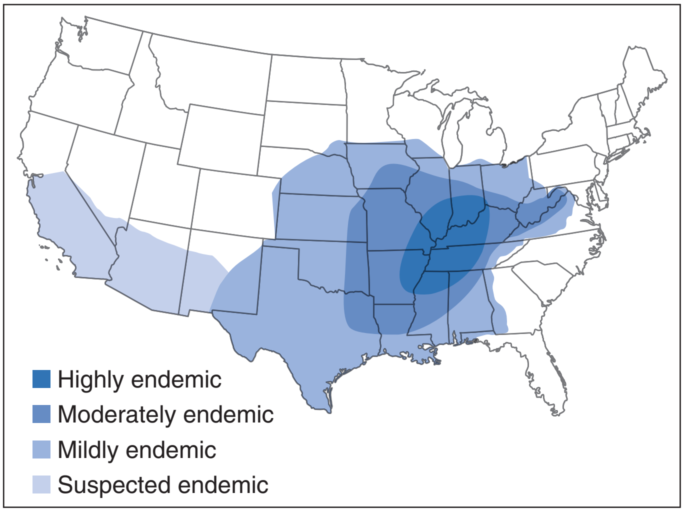


Figure II-5-9. *Histoplasma* Endemic Region

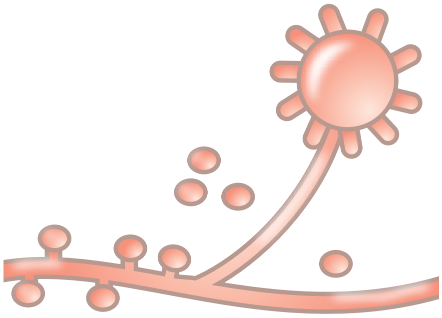


Figure II-5-10. *Histoplasma* Environmental Form

Tissue form: small intracellular yeasts with narrow neck on bud; **no capsule**

- **Facultative intracellular parasite found in reticuloendothelial cells (tiny; can get 30 or so in a human cell)**

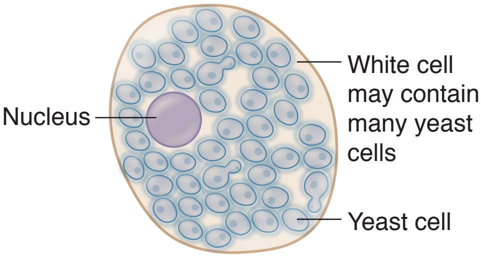


Figure II-5-11. Human RES Cell

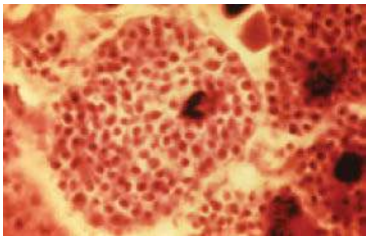


Figure II-5-12. *Histoplasma Capsulatum* Yeast Cells within an RES Cell

Diseases include **fungus flu** (a pneumonia):

- Asymptomatic or acute (but self-resolving) pneumonia with flu-like symptomatology
- **Hepatosplenomegaly may be present** even in acute pulmonary infections (facultative intracellular RES)
- Very common in summer in endemic areas: children or newcomers (80% of adults are skin-test positive in some areas)
- Lesions tend to **calcify as they heal**
- Relapse potential increases with T cell immunosuppression
- **Disseminated infections**: mucocutaneous lesions common; also common **in AIDS** patients in endemic area

Treatment is itraconazole for mild disease and amphotericin B for severe disease.

Coccidioides Immitis

Dimorphic fungus

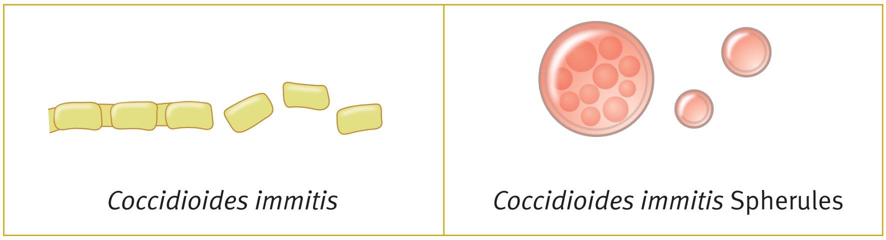


Figure II-5-13. *Coccidioides Immitis*

Environmental form: hyphae breaking up into **arthroconidia** found **in desert sand**

- Endemic region: **Southwestern United States**—Southern **California** (especially San Joaquin Valley), Arizona, New Mexico, Texas, Nevada
- Arthroconidia are inhaled, round up, and enlarged, becoming spherules inside, which the cytoplasm walls off, forming endospores

Tissue form: spherules with endospores

Disease: **Valley fever** (asymptomatic to **self-resolving pneumonia**)

- Good prognostic signs: **desert bumps** (erythema nodosum) and arthritis
- Very common in endemic region
- **Pulmonary lesions tend to calcify** as they heal
- **Systemic infection is a problem in AIDS and immunocompromised patients** in endemic regions (meningitis, mucocutaneous lesions)
- Tend to **disseminate in third trimester of pregnancy**

Treatment is an azole (itraconazole) for mild to moderate disease and amphotericin B for severe disease.

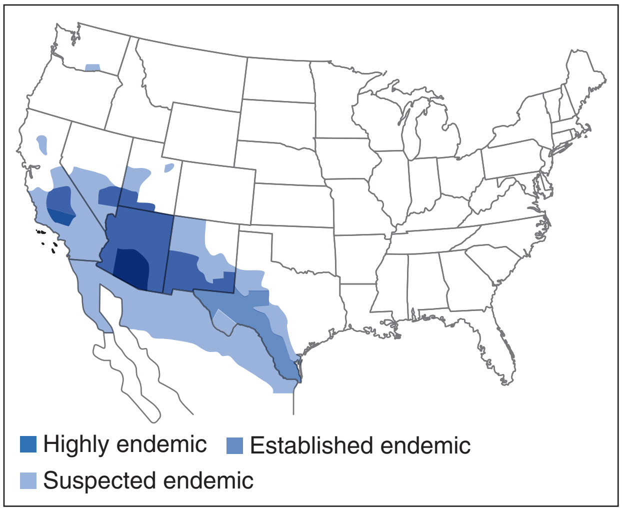


Figure II-5-14. *Coccidioides* Endemic Region

Blastomyces Dermatitidis

Dimorphic fungus

Environmental form: **hyphae** with **nondescript conidia** (i.e., no fancy arrangements)

- No definitive association, although may be associated with **rotting wood** such as beaver dams
- Endemic region: Upper Great Lakes, Ohio, Mississippi River beds, plus southeastern seaboard of the U.S. and northern Minnesota into Canada

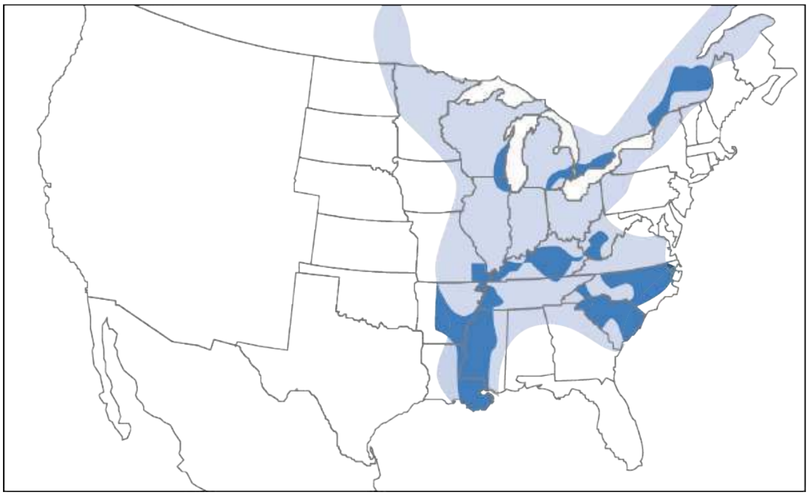


Figure II-5-15. Blastomycosis Endemic Region

Tissue form: broad-based budding yeasts and a double refractile cell wall (not capsule)

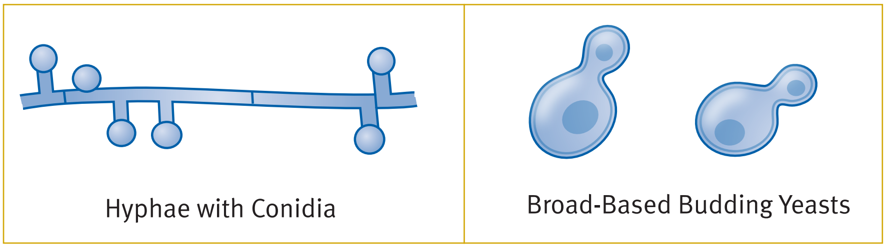


Figure II-5-16. *Blastomyces Dermatitidis*

Paracoccidioides Brasiliensis

Disease: Blastomycosis

- Acute and chronic pulmonary disease
- Considered less likely to self-resolve than *Histoplasma* or *Coccidioides*, so many physicians will treat even acute infections
- Disseminated disease

Treatment is itraconazole (mild/moderate disease) and amphotericin B (severe/systemic disease).

Dimorphic fungus

Environmental form: septate hyphae with intercalated chlamydoconidia

Endemic region: South and Central America (highest incidence Brazil)

Areas with high humidity, moderate temperature, heavy vegetation, and acidic soil

Tissue form: round yeast cells with double refractory walls and multiple buds

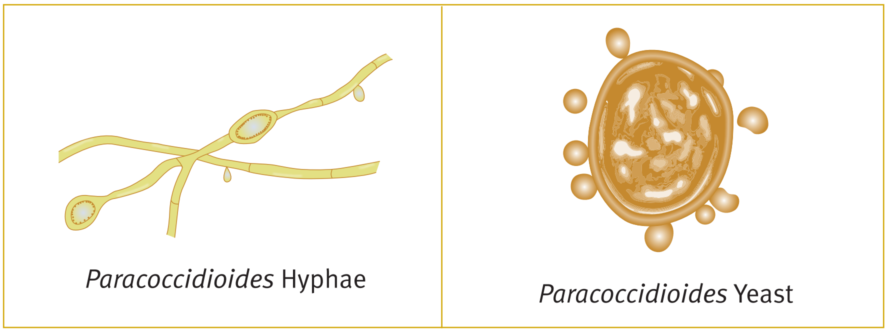


Figure II-5-17. *Paracoccidioides*

Disease. Most infections are subclinical.

- Acute (juvenile) form: flu-like symptoms; lymphadenopathy and hepatosplenomegaly; skin lesions
- Chronic (adult) form: primary pulmonary infection; pulmonary fibrosis, bullae and emphysematous changes (35% of patients); oral and cutaneous lesions

Treatment is typically itraconazole; for severe disease nonresponsive to other antifungals, consider amphotericin B.

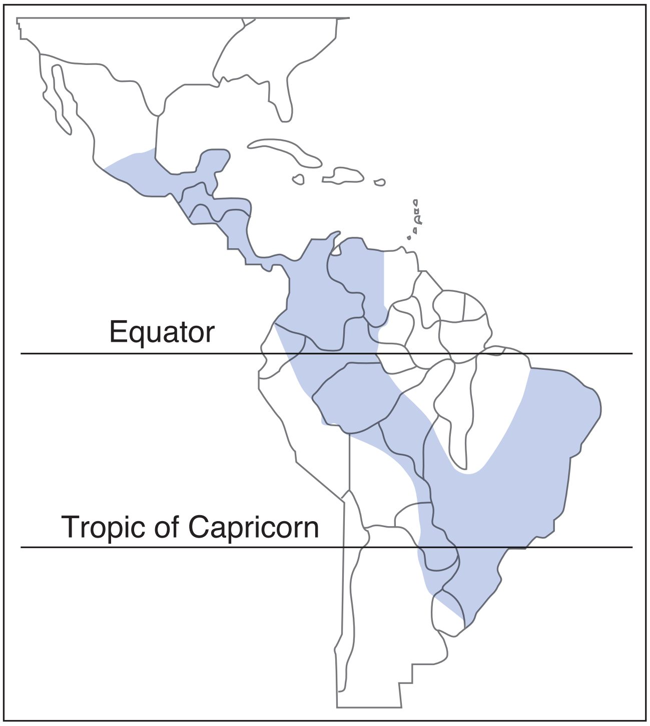


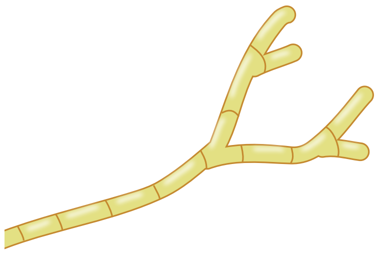
Figure II-5-18. *Paracoccidioides* Endemic Region

Opportunistic Fungi

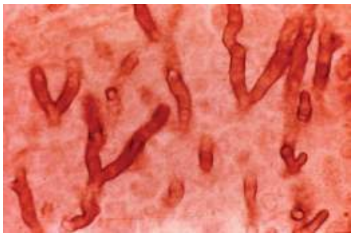
Aspergillus fumigatus

Monomorphic filamentous fungus

- **Dichotomously branching**
- **Generally acute angles**
- **Frequent septate hyphae with 45° angles**
- One of our **major recyclers**: compost pits, moldy marijuana



Aspergillus Showing Monomorphic Filamentous Fungus



Aspergillus fumigatus

Figure II-5-19. Aspergillus

Disease. Predisposing conditions include the following:

- **Allergic bronchopulmonary aspergillosis (ABPA)**/asthma, cystic fibrosis (growing in mucous plugs in the lung but not penetrating the lung tissue)
- **Fungus ball**: free in preformed lung cavities (surgical removal to reduce coughing, which may induce pulmonary hemorrhage)
- **Invasive aspergillosis**/severe neutropenia, CGD, CF, burns
  - Invades tissues causing infarcts and hemorrhage
  - Nasal colonization, leading to **pneumonia or meningitis**
  - **Cellulitis**/in burn patients; may also disseminate

Treatment is voriconazole for invasive aspergillosis and aspergilloma; glucocorticoids and itraconazole for ABPA.

Candida albicans (and other species of *Candida*)

- **Yeast** endogenous to our mucous membrane normal flora
- *C. albicans* yeasts form germ tubes at 37 C in serum
- Forms **pseudohyphae** and **true hyphae** when it invades tissues (nonpathogenic *Candida* do not)

Diseases/Predisposing Conditions

- **Perlèche**: crevices of mouth/malnutrition
- **Oral thrush**/prematurity, antibiotic use, immunocompromised host, AIDS
- **Esophagitis**/antibiotic use, immunocompromised host, AIDS
- **Gastritis**/antibiotic use, immunocompromised host, AIDS
- **Septicemia** (with endophthalmitis and macronodular skin lesions)/ immuno-compromised, cancer and intravenous patients
- **Endocarditis** (with transient septicemias)/**intravenous drug abusers**
- **Cutaneous infections**/obesity and infants; patients with rubber gloves
- **Yeast vaginitis**/particularly a problem in diabetic women
- Chronic mucocutaneous candidiasis/endocrine defects; anergy to *Candida*

Diagnosis is made with **KOH** (pseudohyphae, true hyphae, budding yeasts) and **septicemia** (culture lab identification: biochemical tests/formation of germ tubes).

Treatment is topical or oral imidazole; nystatin; amphotericin B or fluconazole for disseminated disease.

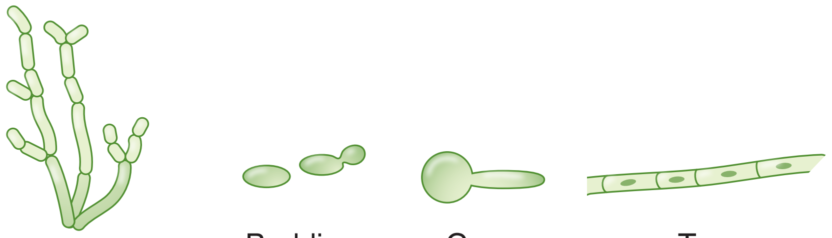


Figure II-5-20. *Candida albicans*

Cryptococcus neoformans

Encapsulated yeast (monomorphic)

Environmental Source: soil enriched with pigeon droppings

Disease. Predisposing conditions include **meningitis/Hodgkins** and **AIDS (the dominant meningitis)**, as well as **acute pulmonary infection** (usually asymptomatic)/**pigeon breeders**.

Diagnosis of meningitis is made with CSF.

- Detect capsular antigen in CSF (by latex particle agglutination or counter immunoelectrophoresis)
- India ink mount (misses 50%) of CSF sediment to find budding yeasts with capsular “halos”
- Cultures (urease positive yeast)

Treatment is AMB+5FC (flucytosine) until afebrile and culture negative (minimum of 10 weeks), then fluconazole.

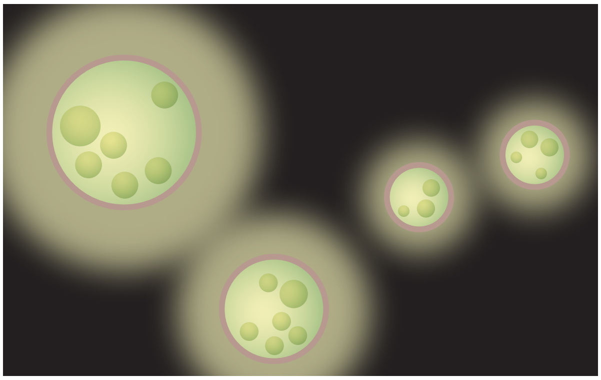


Figure II-5-21. Cryptococcus Neoformans

Mucor, Rhizopus, Absidia (Zygomycophyta)

Nonseptate filamentous fungi

Environmental Source: soil; sporangiospores are inhaled

Disease. These fungi penetrate without respect to anatomical barriers, progressing rapidly from sinuses into the brain tissue.

- **Rhinocerebral infection** caused by *Mucor* (or other zygomycophyta) [old names included mucormycosis = phycomycosis = zygomycosis]
- Characterized by paranasal swelling, necrotic tissues, hemorrhagic exudates from nose and eyes, mental lethargy
- Occurs in **ketoacidotic diabetic** and **leukemic patients**

Diagnosis is made with KOH of tissue; broad ribbon-like nonseptate hyphae with about 90° angles on branches.

Treatment is debridement of necrotic tissue and amphotericin B started immediately. Fatality rate is high due to rapid growth and invasion.

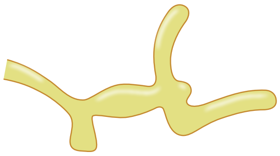


Figure II-5-22. Nonseptate Hyphae with Broad Angles

Pneumocystis jirovecii (formerly *P. carinii*)

Fungus (based on molecular techniques like **ribotyping**)

- **Obligate extracellular parasite**
- **Silver-stained cysts in tissues**

Disease: interstitial pneumonia

- Pneumonia in AIDS patients even with prophylaxis (mean CD4+/mm^3 of 26), malnourished babies, premature neonates, and some other IC adults and kids
- Symptoms: fever, cough, shortness of breath; sputum nonproductive except in smokers
- Serum leaks into alveoli, producing an exudate with foamy or **honeycomb appearance on H&E stain** (silver stain reveals the holes in exudate are actually the cysts and trophozoites, which do not stain with H&E)
- X-ray: **patchy infiltrative** (ground glass appearance); lower lobe periphery may be spared

Diagnosis is made with silver-staining cysts in bronchial alveolar lavage fluids or biopsy.

Treatment is trimethoprim/sulfamethoxazole for mild disease and dapsone for moderate/severe disease.

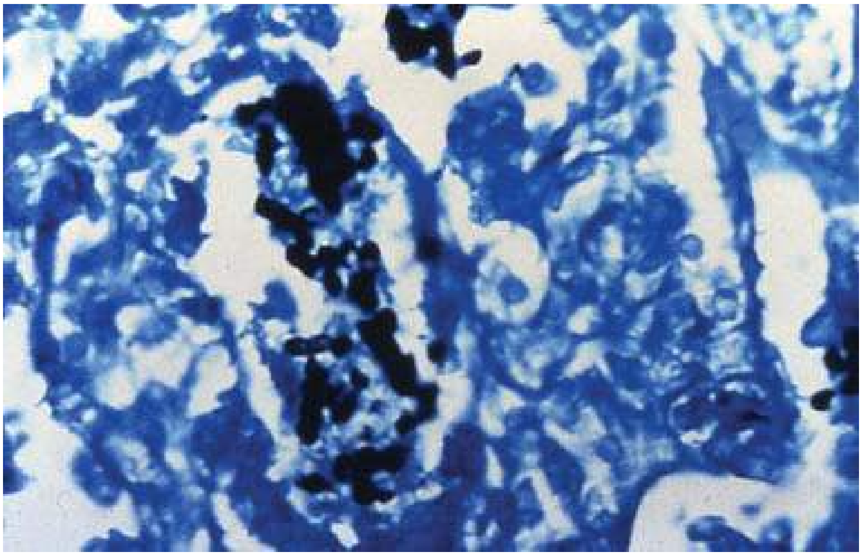


Figure II-5-23. *Pneumocystis, Silver Stain, Exudate*

Note

Fungi are starting to develop **drug resistance** by mechanisms analogous to those seen in bacteria.

- Resistance to azoles becoming widespread
- *Aspergillus, Candida, Cryptococcus*

Medical Parasitology 6

Learning Objectives

- ❑ Demonstrate understanding of classification of parasites
- ❑ Use knowledge of important protozoan parasites
- ❑ Answer questions about important metazoan parasites

CLASSIFICATION OF PARASITES

Medical parasitology is the study of the invertebrate animals and the diseases they cause. Parasites are classified as protozoans or metazoans.

Table II-6-1. Protozoa versus Metazoa

	Protozoa	Metazoa
Complexity	Single-celled	Multicellular
Onset of clinical symptoms	Days to weeks	Typically >1 month
Diagnostic forms	Cysts and trophozoites	Eggs
Elevated immune levels	Typically neutrophils	Eosinophils

The tables list the protozoan and metazoan classifications. The most important organisms in the United States are set in **boldface**.

Table II-6-2. Protozoans

Common Name	Amebae	Flagellates	Apicomplexa
Important genera	***Entamoeba*** *Naegleria* *Acanthamoeba*	LUMINAL (GUT, UG) ***Trichomonas*** ***Giardia*** HEMOFLAGELLATES *Leishmania* *Trypanosoma*	BLOOD/TISSUE ***Plasmodium*** ***Toxoplasma*** *Babesia* INTESTINAL ***Cryptosporidium*** *Cystoisospora*

Table II-6-3. Metazoans: Worms*

Phylum	Roundworms	Flat worms (Platyhelminthes)	
Class **Common name**	**Nematodes**** Roundworms	**Trematodes** Flukes	**Cestodes** Tapeworms
Genera	***Necator*** ***Enterobius*** Wuchereria/*Brugia* ***Ascaris*** and ***Ancylostoma*** *Toxocara, Trichuris & Trichinella* *Onchocerca* *Dracunculus* *Eye worm (Loa loa)* *Strongyloides*	*Fasciola* *Fasciolopsis* *Paragonimus* *Clonorchis* *Schistosoma*	*Diphyllobothrium* *Hymenolepis* *Taenia* *Echinococcus*

* Metazoans also include the Arthropoda, which serve mainly as intermediate hosts (the crustaceans) or as vectors of disease (the Arachnida and Insecta).

** Nematodes mnemonic.

Hosts

The infected host is classified as:

- **Intermediate**: host in which **larval or asexual stages develop**
- **Definitive**: host in which **adult or sexual stages occur**

Vectors

Vectors are **living transmitters** (e.g., a fly) of disease and may be:

- **Mechanical**: transport parasite but there is no development of parasite in the vector
- **Biologic**: some stages of life cycle occur

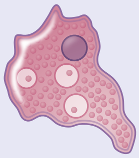
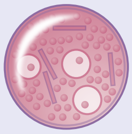
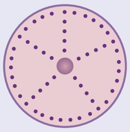
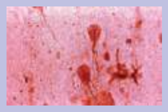
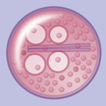
Life Cycle Forms

Life cycle forms may be:

- **Infectious**, e.g., cysts in protozoan infections, eggs in helminth infections
- **Diagnostic**, e.g., trophozoite in protozoan infections, eggs/worm in helminth infection

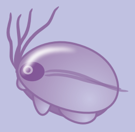
IMPORTANT PROTOZOAN PARASITES

Table II-6-4. Protozoan Parasites

Species	Disease/Organs Most Affected	Form/Transmission	Diagnosis	Treatment
Entamoeba histolytica	*Amebiasis:* dysentery **Inverted flask**-shaped lesions in large intestine with extension to peritoneum and liver, lungs, brain, heart **Blood and pus** in stools **Liver abscesses**	Cysts Fecal-oral transmission: water, fresh fruits, and vegetables	Trophozoites or cysts in stool:   Serology: Nuclei have sharp central karyosome and fine chromatin “spokes.” 	Metronidazole followed by paromomycin
Giardia lamblia	Giardiasis: Ventral sucking disk attaches to lining of duodenal wall, causing **a fatty, foul-smelling diarrhea** (diarrhea → *malabsorption* duodenum, jejunum)	Cysts Fecal (human, beaver, muskrat, etc.), oral transmission (water, food, day care, oral-anal sex)	Pear-shaped trophozoites with bilobed nuclei or cysts in stool, or fecal antigen test   “Falling leaf” motility	Metronidazole
Cryptosporidium **sp.** *C. parvum*	Cryptosporidiosis: transient diarrhea in healthy hosts; severe, immunocompromised hosts	Cysts Undercooked meat, water; not killed by chlorination	**Acid-fast oocysts in stool:** Biopsy shows dots (cysts) in intestinal glands 	Nothing is 100% effective; nitazoxanide, paromomycin, or azithromycin is drug of choice

(*Continued*)

Table II-6-4. Protozoan Parasites (*Cont'd*)

Species	Disease/Organs Most Affected	Form/Transmission	Diagnosis	Treatment
Cystoisospora belli	Transient diarrhea in AIDS patients; diarrhea mimics giardiasis malabsorption syndrome	Oocysts Ingestion Fecal-oral	**Acid-fast, elliptical oocysts in stool;** contain 2 sporocysts each with 4 sporozoites	TMP-SMX or pyrimethamine/ sulfadiazine
Cyclospora cayetanensis	Self-limited diarrhea in immunocompetent; prolonged, severe **diarrhea in AIDS** patients	Oocysts, water, contaminated imported food	Fecal; **acid-fast, spherical oocysts;** contain 2 sporocysts each with 2 sporozoites; UV fluorescence	TMP-SMX
Microsporidia (6 genera)	Microsporidiosis: persistent, debilitating **diarrhea in AIDS** patients; other spp → neurologic, hepatitis, disseminated	Spores ingested	**Gram (+), acid-fast spores** in stool or biopsy material	None proven to be effective
Trichomonas vaginalis (urogenital)	Trichomoniasis: often asymptomatic or **frothy vaginal discharge** Colpitis macularis	Trophozoites **Sexual**	Motile trophozoites in methylene blue wet mount; **corkscrew motility** 	**Metronidazole**

Free Living Ameba

- Occur in water or soil (*Naegleria, Acanthamoeba*)
- Occur in contact lens saline solutions (*Acanthamoeba*): cysts from dust contaminate

Table II-6-5. Free Living Ameba That Occasionally Infects Humans

Species	Disease/Locale	Form/Transmission	Diagnosis	Treatment
Naegleria	**Primary amebic meningoencephalitis** (PAM): severe **prefrontal headache,** nausea, high fever, **altered sense of smell**; often fatal	Free living ameba picked up while swimming or **diving in very warm fresh water**	**Motile trophozoites in CSF** Culture on plates seeded with gram-negative bacteria; ameba will leave trails	Amphotericin B (rarely successful)
Acanthamoeba	**Keratitis; granulomatous amebic encephalitis** (GAE) in immunocompromised patients; insidious onset but progressive to death	Free living ameba in contaminated **contact lens solution (airborne cysts)** Not certain for GAE: inhalation or contact with contaminated soil or water	Star-shaped cysts on biopsy; rarely seen in CSF culture as above	Keratitis: topical miconazole and propamidine isethionate; GAE: ketoconazole, sulfamethazine (rarely successful)

Plasmodium Species

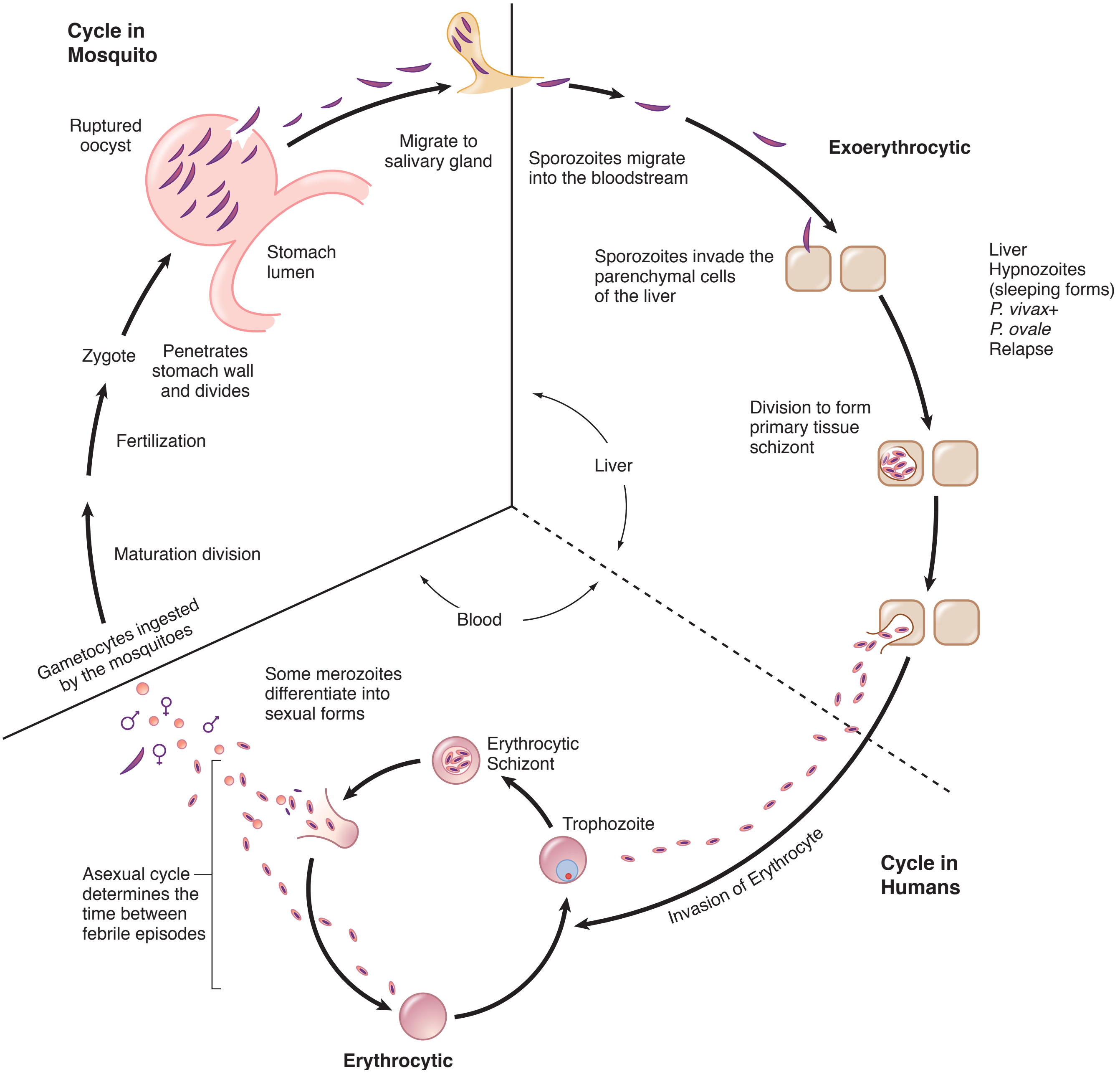


Figure II-6-1. *Plasmodium* Life Cycle

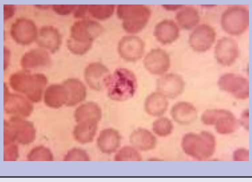
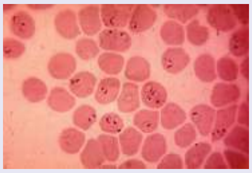
Each *Plasmodium* has 2 distinct hosts: **vertebrate** (such as the human) where asexual phase (schizogony) takes place in the liver and red blood cells, and **arthropod** host (*Anopheles* mosquito) where gametogony (sexual phase) and sporogony take place.

- Cause disease by various mechanisms, including metabolism of hemoglobin and lysis of infected cells leading to anemia and agglutination of infected red blood cells

- Cause paroxysms (chills, fever spike, and malarial rigors) when infected red blood cells are lysed, liberating a new crop of merozoites

HbS heterozygote—selective protection against *P. falciparum*. Duffy blood group Ag—receptor for *P. vivax*. Other abnormal hemoglobins (e.g., thalassemia) are indigestible to all *Plasmodium* spp.

Table II-6-6. *Plasmodium* Species

Species	Important Features	Blood Smears	Liver Stages	Treatment**
Plasmodium vivax	48-hour fever spikes	Enlarged host cells; ameboid trophozoites 	Persistent **hypnozoites** **Relapse***	Chloroquine, then **primaquine**
Plasmodium ovale (2 substrains)	48-hour fever spikes	Oval, jagged, infected RBCs	Persistent hypnozoites Relapse	Chloroquine, then primaquine
Plasmodium malariae	72-hour fever spikes; recrudescence*	Bar and band forms; rosette schizonts	No persistent stage*; recrudescence*	Chloroquine (no radical cure necessary)
Plasmodium falciparum	Irregular fever spikes; causes **cerebral malaria**	**Multiple ring forms crescent-shaped gametes** 	No persistent stage*; recrudescence	• Chloroquine for susceptible, non-severe cases • Atovaquone-proguanil, artemether-lumefantrine, mefloquine for resistant, non-severe cases • Quinine/quinidine for severe cases
Plasmodium knowlesi	24-hour fever spikes	Similar to *P. malariae*	No persistent stage	Chloroquine

* Recrudescence is a reoccurrence of symptoms from low levels of organisms remaining in red cells. Relapse is a return of clinical symptoms from liver stages (hypnozoites).

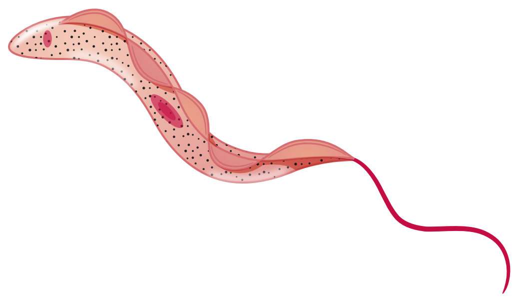
** **Treatment:**

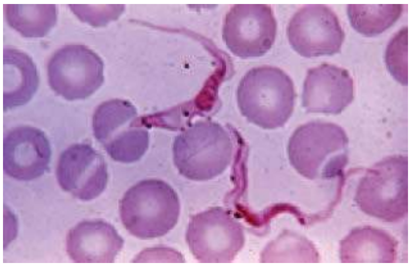
1. Suppressive (to avoid infection)
2. Therapeutic (eliminate erythrocytic)
3. Radical cure (eliminate hypnozoites)
4. Gametocidal (destruction of gametocytes)

Successful treatment is accomplished with chloroquine followed by primaquine. Chloroquine therapy is suppressive, therapeutic, and gametocidal, whereas primaquine eliminates the exoerythrocytic form.

Hemoflagellates (Trypanosomes and Leishmanias)

Hemoflagellates infect blood and tissues.





Trypomastigote in blood smear

Figure II-6-2. Trypomastigote

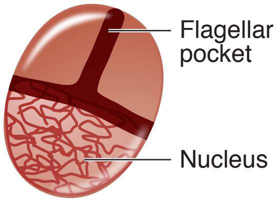


Figure II-6-3. Amastigote

Trypanosomes are found in **human blood** as trypomastigotes with flagellum and undulating membrane and in **tissue** as **amastigotes** (**oval cells having neither the flagellum nor undulating membrane**).

Leishmanias are always found as amastigotes inside macrophages.

Table II-6-7. Hemoflagellates

Species	Disease	Vector/Form/ Transmission	Reservoirs	Diagnosis	Treatment
*Trypanosoma cruzi**	• **Acute**: **Chagas disease** (American trypanosomiasis); Mexico, South/ Central America; swelling around eye (**Romaña sign**); myocarditis and meningoencephalitis • **Chronic**: dilated cardiomyopathy, megacolon, achalasia	**Reduviid bug (kissing or cone bug**; genus *Triatoma*) passes trypomastigote (flagellated form) **in feces**; scratching implants in mucosa	• Cats, dogs, armadillos, opossums • Poverty housing	Blood films, **trypomastigotes**	Benznidazole or nifurtimox
Trypanosoma brucei gambiense Trypanosoma b. rhodesiense	**African sleeping sickness** (African trypanosomiasis) **Antigenic variation**	Trypomastigote in saliva of **tsetse fly** contaminates bite	Humans, some wild animals	• Trypomastigotes in blood films, CSF • High immunoglobulin levels in CSF	Acute: suramin Chronic: melarsoprol
*Leishmania donovani*** complex	**Visceral leishmaniasis**	**Sandfly** bite	Urban: humans Rural: rodents and wild animals	**Amastigotes in macrophages** in bone marrow, liver, spleen	Stibogluconate sodium (from CDC)
Leishmania (About 15 different species)	Cutaneous leishmaniasis (Oriental sore, etc.)	**Sandfly** bite	Urban: humans Rural: rodents and wild animals	**Amastigotes in macrophages** in cutaneous lesions	Stibogluconate sodium
Leishmania braziliensis complex	Mucocutaneous leishmaniasis	**Sandfly** bite	Urban: humans Rural: rodents and wild animals	Same	Stibogluconate sodium

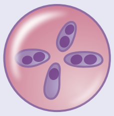
* *T. cruzi:* An estimated 1/2 million Americans are infected, creating some risk of transfusion transmission in U.S. In babies, acute infections often serious involving CNS.

In older children and adults, mild acute infections but may become chronic with the risk of development of cardiomyopathy and heart failure.

** *Leishmania* all: Intracellular, sandfly vector, stibogluconate.

Miscellaneous Apicomplexa Infecting Blood or Tissues

Table II-6-8. Miscellaneous Apicomplexa Infecting Blood or Tissues

Species	Disease/Locale of Origin	Transmission	Diagnosis	Treatment
Babesia	Babesiosis (hemolytic, **malaria-like**)	***Ixodes* tick** **Coinfections with** ***Borrelia***	Giemsa stain of thin smear or hamster inoculation; small rings, **maltese cross**, tetrad in red blood cells 	Clindamycin + quinine
Babesia microti, WA1, & MO1 strains	Same range as Lyme: NE, N Central, California, and NW United States			
Toxoplasma gondii	**Cat** (**essential definitive** host); many other animals (intermediate host) Mode: raw meat in U.S. (#1 is pork) and contact with cat feces	Serology High IgM or rising IgM acute infection		**Pyrimethamine + sulfadiazine**

Toxoplasmosis

Diseases. In **healthy individuals**, *Toxoplasma* acquired after birth is often asymptomatic or a mild, nonspecific flu-like illness with lymphadenopathy and fever (heterophile-negative mononucleosis). Once infected, as immunity develops, bradyzoites encyst, but generally remain viable as evidenced by a positive antibody titer.

Toxoplasma acquired as a primary infection **during pregnancy** often presents with flu-like illness/heterophile-negative mononucleosis; the fetus may be infected.

- If *Toxoplasma* **crosses placenta early**, severe congenital infection (intracerebral calcifications, chorioretinitis, hydro- or microcephaly or convulsions) may occur; if **crosses placenta later**, infection may be inapparent but may lead to progressive blindness in the child later in life (teens)
- Maternal antibodies (secondary infection) protect the fetus during pregnancy, even if mother is re-exposed during pregnancy

In **AIDS** patients, *Toxoplasma* is the leading cause of focal CNS disease. A brain scan will describe ring-enhancing lesions. Unless prophylactic drugs are given, AIDS patients who are seropositive for *Toxoplasma* will have reactivational infections.

IMPORTANT METAZOAN PARASITES

Nematodes

The nematodes are the roundworms. They cause a variety of diseases (e.g., pinworms, whipworms, hookworms, trichinosis, threadworms, filariasis).

- Round unsegmented bodies
- Transmitted by:
  - Ingestion of eggs (*Enterobius, Ascaris, Trichuris*) or meat containing larvae (*Trichinella*)
  - Direct invasion of skin by larval forms (*Necator, Ancylostoma, Strongyloides*)
  - Infection involving insects transmitting the larvae with bites (*Wuchereria, Loa loa, Mansonella, Onchocerca*)

Table II-6-9. Round Worms (Nematodes) Transmitted by Eggs

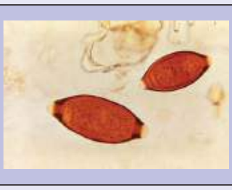
Species	Disease/Organs Most Affected	Form/ Transmission	Diagnosis		Treatment
Enterobius vermicularis Most frequent helminth parasite in U.S.	**Pinworm** Large intestine, nocturnal perianal itching	**Eggs**/person to person **Autoinfection**	• Cellophane tape prep of perianal area • Ova have flattened side with larvae inside		Pyrantel, **albendazole** Treat entire family
Trichuris trichiura	**Whipworm** Cecum, appendicitis, and rectal prolapse	**Eggs** Ingested	**Barrel-shaped eggs with bipolar plugs** in stools		Albendazole
Ascaris lumbricoides Most common helminth worldwide Largest roundworm	**Ascariasis** Ingest egg → larva migrate thru lungs (cough) and mature in small intestine; may obstruct intestine or bile duct	**Eggs** Ingested	**Bile stained, knobby eggs** Adults **6–12"** roundworms		Supportive therapy during pneumonitis; surgery for ectopic migrations; albendazole
Toxocara canis or cati (dog/cat ascarids)	**Visceral larva migrans** Larvae wander aimlessly until they die, cause inflammation	**Eggs** Ingested/from handling puppies or from eating dirt in yard (pica)	Clinical findings and serology		Albendazole; self-limiting in most cases

Table II-6-10. Roundworms (Nematodes) Transmitted By Larvae

Species	Disease/Organs	Form/Transmission	Diagnosis	Treatment
Necator americanus New World hookworm	**Hookworm** infection Lung migration → pneumonitis bloodsucking → anemia	Filariform **larva penetrates intact skin of bare feet**	Fecal larvae (up to 13 mm) and ova: oval, transparent with 2–8 cell-stage visible inside Occult blood fecal may be present	**Albendazole** and iron therapy
Ancylostoma braziliense Ancylostoma caninum (dog and cat hookworms)	**Cutaneous Larva Migrans**/intense skin itching	Filariform larva penetrates intact skin but cannot mature in humans	Usually a presumptive diagnosis; exposure	Thiabendazole Topical corticosteroids
Strongyloides stercoralis	**Threadworm** strongyloidiasis: Early: pneumonitis, abdominal pain, diarrhea Later: malabsorption, ulcers, bloody stools	Filariform **larva penetrates intact skin** **Autoinfection** leads to indefinite infections unless treated	Larvae in stool, serology	Ivermectin
Trichinella spiralis	Trichinosis: larvae encyst in muscle → pain	**Viable encysted larvae in meat** are consumed; wild game meat (bear meat)	Muscle biopsy; clinical findings: **fever, myalgia, splinter hemorrhages, eosinophilia**	Steroids for severe symptoms and albendazole

Table II-6-11. Filarial Nematodes

Species	Disease	Transmission/ Vector	Diagnosis	Treatment
Wuchereria bancrofti; Brugia malayi	Elephantiasis; tropical pulmonary eosinophilia	Mosquito	Microfilariae in blood, eosinophilia	Surgery, ivermectin and diethylcarbamazine
Loa loa (African eye worm)	Pruritus, Calabar swellings	*Chrysops* Mango flies, deer flies	Microfilariae in blood, eosinophilia	Surgical removal of worms; diethylcarbamazine or ivermectin
Onchocerca volvulus	River blindness, itchy “leopard” rash	Blackflies	Skin snips from Calabar swellings	Surgical removal of worms; diethylcarbamazine or ivermectin
Dracunculus medinensis (guinea worm, fiery serpent)	Creeping eruptions, ulcerations, rash	Drinking water with infected copepods	Increased IgE; worm eruption from skin	Slow, cautious worm removal with stick

Trematodes

Trematodes are commonly called **flukes**. The first intermediate hosts are **snails**.

- Leaf-shaped worms, generally flat and fleshy
- Hermaphroditic (except for *Schistosoma,* which is either male or female)
- Have complicated life cycles occurring in 2 or more hosts
- Have **operculated eggs** (except for *Schistosoma*), which contaminate water, perpetuating the life cycle; also used to diagnose infections

Table II-6-12. Trematode (Fluke) Diseases

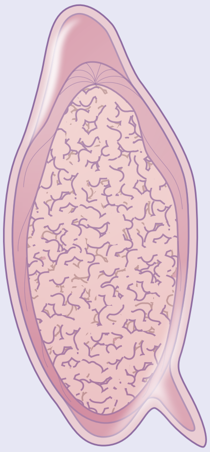
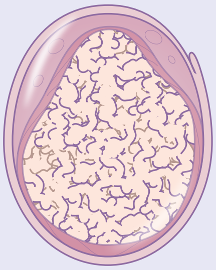
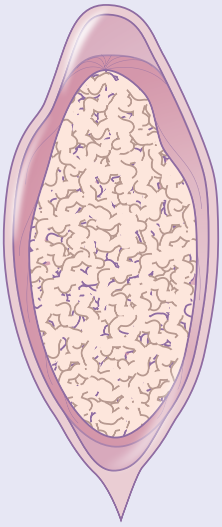
Organism	Common Name	Reservoir Host	Acquisition	Progression in Humans	Important Ova	Treatment
S. mansoni *Schistosoma japonicum*	**Intestinal schistosomiasis**	Cats, dogs, cattle, etc.	Contact with water; **skin penetration**	Skin penetration (itching) → mature in veins of mesentery → eggs cause granulomas in liver (liver enlargement in chronic cases) Clay pipe-stem fibrosis of portal vein	 	Praziquantel
Schistosoma haematobium	**Vesicular schistosomiasis**	Primates	Contact with water; **skin penetration**	Skin penetration (itching) → mature in bladder veins; chronic infection has high association with **bladder carcinoma in Egypt and Africa**		Praziquantel

Table II-6-12. Trematode (Fluke) Diseases (*Cont'd*)

Organism	Common Name	Reservoir Host	Acquisition	Progression in Humans	Important Ova	Treatment
Nonhuman schistosomes	**Swimmer's itch**	Birds (Great Lakes U.S.)	Contact with water; **skin penetration**	Penetrate skin, producing **dermatitis** without further development in humans; itching is most intense at 2 to 3 days		Trimeprazine Calamine Sedatives
Clonorchis sinensis	**Chinese liver fluke**	Dogs, cats, humans	Raw fish ingestion	Serum-like sickness	Operculated eggs	Praziquantel
Fasciola hepatica	Sheep liver fluke	Sheep	Watercress	Hepatitis	Operculated eggs	Praziquantel
Fasciolopsis buski	Giant intestinal fluke	Pigs	Water chestnuts	Diarrhea ± malabsorption syndrome	Operculated eggs	Praziquantel
Paragonimus westermani*	Lung fluke	Pigs/dogs	Ingestion of raw crab/ crayfish from Asia, Africa, South America	TB-like disease: hemoptysis, night sweats	Operculated eggs in sputum	Praziquantel

* *Paragonimus kellicotti* causes similar disease from ingestion of raw crayfish in Midwest United States

Cestodes

The cestodes are the tapeworms. For the most part, they have complex life cycles involving extraintestinal larval forms in intermediate hosts. When humans are the intermediate host, these infections are more serious than the intestinal infections with adult tapeworms.

- Have 3 parts: a **head** or scolex, a "**neck**" that produces the **proglottids** (together, the neck and proglottids are called the strobila), and the **segments** or proglottids that mature as they move away from the scolex
- Hermaphroditic, with each proglottid developing both male and female reproductive organs, and mature eggs developing in the most distal proglottids
- Adhere to the mucosa via the scolex, which is knobby-looking and has suckers or a sucking groove
- Have no gastrointestinal tract; instead, absorb nutrients from the host's gastrointestinal tract
- Diagnosed by finding eggs or proglottids in the feces

Gastrointestinal Cestodes (Tapeworms)

Table II-6-13. Gastrointestinal Cestodes (Tapeworms)

Cestode*	Form/Transmission	Humans Are	Disease/Organ Involvement/ Symptoms (Sx)	Diagnosis	Treatment
Taenia saginata **(beef tapeworm)** IH: cattle DH: humans	Rare beef containing the **cysticerci** is ingested	DH*	**Intestinal tapeworm**/ small intestine Sx: asymptomatic or vague abdominal pains	**Proglottids** or **eggs** in feces	Praziquantel
Taenia solium **(pork tapeworm)** IH: swine; Rarely: humans DH: humans; developing and Slavic countries	Water, vegetation, food contaminated with **eggs** **Autoinfection**	IH*	**Cysticercosis**/eggs → larva develop in brain, eye, heart, lung, etc. Adult-onset epilepsy (seizures)	Biopsy	Praziquantel: surgery in some sites
	Rare/raw pork containing the **cysticerci** is ingested by humans	DH	**Intestinal tapeworm** Sx: as for *Taenia saginata*	**Proglottids** or **eggs** in feces	Praziquantel
Diphyllobothrium latum **(fish tapeworm)** IH (2): crustaceans → fish; rare: humans DH: humans/mammals; cool lake regions	Drinking pond water w/→ copepods (crustaceans) carrying the **larval** forms or frog/snake poultices	IH	**Sparganosis**/larvae penetrate intestinal wall and encyst	Biopsy	Praziquantel
	Rare, raw pickled fish → containing a **sparganum**	DH	**Intestinal tapeworm** (up to 10 meters)/ small intestine **megaloblastic anemia**	Proglottids or eggs in feces	Praziquantel
Echinococcus granulosus IH: herbivores; rare: humans DH: carnivores in sheep-raising areas	Ingestion of eggs	IH	**Hydatid cyst disease**/liver and lung where cysts containing brood capsules develop	Imaging; serology	Surgery; albendazole
Echinococcus multilocularis IH: rodents DH: canines and cats; northern areas	Ingestion of eggs	IH	**Alveolar hydatid cyst disease**	Imaging; serology	Surgical resection

* Definitive host = adult tapeworm develops in; intermediate host = cysticerci or larvae develop in; cysticerci = encysted larvae found in intermediate host. Common name is in parentheses.

Clinical Infectious Disease 7

Learning Objectives

- ❏ Answer case-based questions related to infectious disease

These charts are designed for self-study after the organisms have all been reviewed in class. They represent the basics used in clinical scenarios on the USMLE.

Cover the last column on each chart and write the causal agent(s) on paper. Then think about how the organism causes disease. Is there a major virulence factor?

Abbreviations Used

→ means progressing on to

~ means about or approximately

HIV+ = patient with known human immunodeficiency virus infection; can be used for anyone who is infected but often used for those who are HIV+ but do not have full blown AIDS (in other words, CD4+ count >200)

AIDS = acquired immunodeficiency syndrome (CD4+ count <200)

abd = abdominal

CF = cystic fibrosis

CMI = cell-mediated immunity

CGD = chronic granulomatous disease

GU = genitourinary

IC = immunocompromised

Infl'd = inflamed

Infl'n = inflammation

IV = intravenous

mo = month(s)

NF = normal flora

occ = occasional

PMNs = polymorphonuclear leukocytes

pt = patient

RBCs = red blood cells

subQ = subcutaneous

If there are multiple causal agents, at the end of the description there may be a # with the abbreviation "CA." This means you should be able to list that number. If it specifically says "species," you should give species.

Table II-7-1. Diseases of Skin, Mucous Membranes, and Underlying Tissues

Type Infection	Case Vignette/Key Clues	Common Causal Agents
Furuncles, carbuncles	Neck, face, axillae, buttocks	*Staphylococcus aureus*
	Inflamed follicles from neck down	*Pseudomonas aeruginosa* (hot tub folliculitis)
Acne vulgaris	Inflammation of follicles and sebaceous glands	*Propionibacterium acnes*
Impetigo	Initially vesicular; skin erosion; **honey-crusted** lesions; catalase negative organism	*Streptococcus pyogenes*
	Initially vesicular but with longer lasting **bullae**; catalase-positive organism	*Staphylococcus aureus*
Vesicular lesions	Sometimes preceded by neurologic pain	Herpes
SubQ granulomas/ulcers/cellulitis	Tropical fish enthusiasts; granulomatous lesion	*Mycobacterium marinum* (fish tank granuloma)
	Cellulitis following contact with saltwater or oysters	*Vibrio vulnificus*
Mycetoma (swelling with pain, sinus tract formation, yellow granules in exudate)	Solitary or lymphocutaneous lesions, rose gardeners or florists, sphagnum moss	*Sporothrix schenckii* (rose gardener disease)
	Subcutaneous swelling (extremities, shoulders) multiple CA	Bacteria: *Actinomyces, Nocardia*, Fungi: *Madurella, Pseudallescheria, Sporothrix*
	Jaw area, associated with carious teeth, dental extraction, or trauma	*Actinomyces israelii* “lumpy jaw”
Malignant pustule	Pustule → dark red fluid-filled, tumor-like lesion → necrosis → black eschar surrounded by red margin	*Bacillus anthracis*
	Ecthyma gangrenosum (as above)	*Pseudomonas* septicemia
Cellulitis	Blue-green pus, grape-like odor, burns	*Pseudomonas aeruginosa*
	Dermal pain, edema, heat and rapid spread. Red, raised butterfly facial rash	*Streptococcus pyogenes* (Erysipelas)
	Hot inflamed tissues. Deeper tissues from extension of skin lesions or wounds including surgical	Variety of bacteria: *S. aureus*, *S. pyogenes*, gram (–) rods, *Clostridium* and anaerobes

(*Continued*)

Table II-7-1. Diseases of Skin, Mucous Membranes, and Underlying Tissues (*Cont'd*)

Type Infection	Case Vignette/Key Clues	Common Causal Agents
Wounds	Surgical wounds (clean)	*Staphylococcus aureus*
	Surgical wounds (dirty)—list groups	*S. aureus*, *Enterobacteriaceae*, anaerobes
	Trauma—list groups	*Clostridium*, Enterobacteriaceae, *Pseudomonas*
	Shallow puncture wound through tennis shoe sole	*Pseudomonas aeruginosa*
Animal bites	Various	*Pasteurella multocida*
	Human bites, fist fights	*Eikenella corrodens*
	Dog bites	*Capnocytophaga canimorsus*
	Rat bites	*Streptobacillus moniliformis and Spirillum minus*
	Cat scratches resulting in lymphadenopathy with stellate granulomas	*Bartonella henselae*

Table II-7-2. Ear, Nose, Throat, Upper Respiratory System Infections

Type Infection	Case Vignette/Key Clues	Common Causal Agents
Acute otitis media	Red, bulging tympanic membrane, fever 102–103; pain goes away if drum ruptures or if ear tubes are patent. 5 CA	*Streptococcus pneumoniae* *H. influenzae* (often nontypeable, recurs) *Moraxella catarrhalis* RSV Rhinovirus
Otitis externa	Ear pain—list of organisms	Normal flora often involved Often mixed infections: *Staph aureus* (from NF)* *Candida albicans* (from NF)* *Proteus* (water organism) *Pseudomonas* (water)
Malignant otitis externa	Severe ear pain in diabetic; life threatening	*Pseudomonas aeruginosa*
Sinusitis	Sinus pain; low-grade fever	As for acute otitis media
Oral cavitary disease	Painful mouth—overgrowth of spirochetes and fusiform bacteria	*Fusobacterium* and treponemes (normal oral spirochetes)
	Sore mouth with thick white coating (painful red base under); increased risk: premature infants, AIDS, IC pts, pts on antibiotics, vitamin C deficiency	*Candida*
Sore throat	Inflamed tonsils/pharynx, which may be purulent and may develop abscesses; cervical lymphadenopathy, fever, stomach upset; sandpaper rash	*Streptococcus pyogenes* (group A strep) Rash indicates presence of erythrogenic exotoxin A
	White papules with red base on posterior palate and pharynx, fever	Coxsackie A
	Throat looking like Strep with severe fatigue, lymphadenopathy, fever, rash; heterophile (+); Downey type II cells	Epstein-Barr virus
	Low-grade fever with a 1–2 day gradual onset of membranous nasopharyngitis and/or obstructive laryngotracheitis; bull neck from lymphadenopathy; elevated BUN; abnormal ECG; little change in WBC (toxin). Exudate bleeds profusely when dislodged	*Corynebacterium diphtheriae* (diphtheria)
Common cold	Rhinitis, sneezing, coughing; list CA with seasonal peaks	Rhinoviruses (summer–fall) Coronaviruses (winter–spring) Human metapneumovirus Adenovirus, many others

*NF = normal flora

Table II-7-3. Eye Infections

Type Infection	Case Vignette/Key Clues	Common Causal Agents
Eyelid	Bilateral eye lid swelling, ›10% eosinophilia, fever, muscle pain; earlier GI Sx	*Trichinella*
	Stye; 2 CA	*Staphylococcus aureus* *Propionibacterium acnes*
	Unilateral inflammation at bite site often around eye or mouth; travel to Mexico, Central or South America	*Trypanosoma cruzi*
Conjunctivitis neonate	Red itchy eye(s)/pus; onset 2–5 days Red itchy eye(s)/pus; onset 5–10 days	Bacterial pink eye *Neisseria gonorrhoeae* *Chlamydia trachomatis* (serotypes D–K U.S.)
	Neonate with "sticky eye"	*Staphylococcus aureus*
Conjunctivitis (other age groups)	Red itchy eye(s), thin exudate; pain, photophobia	Viral pink eye: adenovirus (more common than bacterial pink eye)
	Red eye, pus; 4 CA	*S. aureus*, group A Strep, *Strep pneumoniae* (all gram [+]) *Haemophilus influenzae*, *H. aegyptius*
	Red eye, pus, presence of inclusion bodies in scrapings; CA with serotypes in U.S.	*Chlamydia trachomatis* serotypes D-K (inclusion conjunctivitis)
	Granulomas and inturned eye lashes, corneal scarring, blindness; CA with serotypes	*Chlamydia trachomatis* serotypes A, B, Ba, C (trachoma)
Chorioretinitis	Neonate or AIDS; 2 CA	*Toxoplasma*, CMV
Retinopathy with keratitis in baby	Mom IV drug abuser	*Treponema pallidum* (congenital syphilis)

Table II-7-4. Cardiac Symptoms

Type Infection	Case Vignette/Key Clues	Common Causal Agents
Acute endocarditis: Chills, fever, arthralgia, myalgia, back pain, acutely ill, Janeway lesions; emboli	Developing a heart murmur; IV drug user	*Staphylococcus aureus*
	Not IV drug user	*Staphylococcus aureus*
Subacute endocarditis: Fever with vague symptoms with insidious onset, fatigue, weakness, weight loss, night sweats, anorexia, myalgias; murmur may have been long present; emboli, splinter hemorrhages	Poor oral hygiene or dental work	*Viridans* streptococci (55% of cases in native hearts)
	Gram neg. endocarditis (normal oral flora)	HACEK organisms *(**H**aemophilus aphrophilus* ***A**ctinobacillus actinomycetemcomitans* ***C**ardiobacterium hominis* ***E**ikenella corrodens* ***K**ingella kingae)*
	Biliary or urinary tract infection GU manipulation in elderly men	*Enterococcus faecalis*
	IV drug user	*Staph. epidermidis* *Aspergillus* (branching ‹45) *Candida* (pseudohyphae) *Pseudomonas* *Viridans* streptococci
Dilated cardiomyopathy	Rural South America	*Trypanosoma cruzi*

Table II-7-5. Middle and Lower Respiratory System Infections

Type Infection	Case Vignette/Key Clues	Most Common Causal Agents
Respiratory difficulty or obstruction	Inflamed epiglottis; patient often 2–3 and unvaccinated	*Haemophilus influenzae* (epiglottitis)
	Infant with fever, sharp barking cough, inspiratory stridor, hoarse phonation	Parainfluenza virus (Croup)
Bronchitis	Wheezy; infant or child ≤5 years	RSV
	>5 years	*Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae*
	With cough >2 weeks, afebrile; >9	*Bordetella pertussis*
Pneumonia **Typical:** high fever, productive cough, diffuse infiltrates	Poorly nourished, unvaccinated baby/child; giant cell pneumonia with hemorrhagic rash	Measles: malnourishment ↑ risk of pneumonia and blindness
	Adults (including alcoholics) #1 CA Rusty sputum, often follows influenza	*Streptococcus pneumoniae*
	Neutropenic pts, burn pts, CGD, CF	*Pseudomonas*
	Foul smelling sputum, aspiration possible	Anaerobes, mixed infection (*Bacteroides, Fusobacterium, Peptococcus*)
	Alcoholic, abscess formation, aspiration, facultative anaerobic, gram-negative bacterium with huge capsule, currant jelly sputum	*Klebsiella pneumoniae*
	Nosocomial, ventilator, post-influenza Abscess formation Gram +, catalase +, coagulase + Salmon-colored sputum	*Staphylococcus aureus*
Atypical: low fever, dry cough, diffuse infiltrates	Pneumonia teens/young adults; bad hacking cough; initially non-productive cough	*Mycoplasma pneumoniae* (most common cause of pneumonia in school age children)
	Atypical with air conditioning exposure especially >50 yr, heavy smoker, drinker	*Legionella* spp.
	Atypical with bird exposure, hepatitis	*Chlamydophila psittaci*
	AIDS patients with staccato cough; "ground glass" x-ray; biopsy: honeycomb exudate with silver staining cysts, progressive hypoxia	*Pneumocystis jirovecii*
Acute respiratory distress	Travel to Far East, winter, early spring, hypoxia	SARS-CoV
	Spring, 4 corners region, exposure to rodents	Hanta virus
Acute pneumonia or chronic cough with weight loss, night sweats, calcifying lesions	Over 55, HIV+, or immigrant from developing country	*Mycobacterium tuberculosis*
	Dusty environment with bird or bat fecal contamination (Missouri chicken farmers), yeasts packed into phagocytic cells	*Histoplasma capsulatum*
	Desert sand, SW U.S.	*Coccidioides immitis*
	Rotting contaminated wood, North and South Carolina	*Blastomyces dermatitidis*

Table II-7-6. Genitourinary Tract Infections

Type Infection	Case Vignette/Key Clues	Most Common Causal Agents
Urethritis	Gram-negative diplococci in PMNs in urethral exudate	*Neisseria gonorrhoeae*
	Culture negative, inclusion bodies	*Chlamydia trachomatis*
	Urease positive, no cell wall	*Ureaplasma urealyticum*
	Flagellated protozoan with corkscrew motility	*Trichomonas vaginalis*
Cystitis	Frequent and painful urination, hematuria, and fever	#1 *E. coli*, other gram-negative enterics, *Pseudomonas, Proteus*
	Young, newly sexually active individual; gram-positive cocci	*Staphylococcus saprophyticus*
Pyelonephritis	As above, with flank pain and prominent fever	*E. coli, Staphylococcus*
Cervicitis	Friable, inflamed cervix with mucopurulent discharge; probes or culture to distinguish	*Neisseria gonorrhoeae* (gram-negative diplococci) *Chlamydia trachomatis* (non-staining obligate intracellular parasite) Herpes simplex (virus)
Vaginal itching, pain, discharge odor	Adherent yellowish discharge, pH ›5, fishy amine odor in KOH, clue cells; gram-negative cells dominate	(Bacterial vaginosis) overgrowth of *Gardnerella vaginalis* and anaerobes
	Vulvovaginitis, pruritis, erythema, discharge: consistency of cottage cheese	*Candida* spp.
	Foamy, purulent discharge, many PMNs and motile trophozoites microscopically (corkscrew motility)	*Trichomonas vaginalis*
Pelvic inflammatory disease	Adnexal tenderness, bleeding, deep dyspareunia, vaginal discharge, fever; tenderness from cervical movement, possibly palpable inflammatory mass on bimanual exam, onset often follows menses	*Neisseria gonorrhoeae* or *Chlamydia trachomatis* or both or a variety of other organisms
Genital lesions	Genital warts	Human papilloma virus (most common U.S. STD), *Treponema pallidum*, molluscum contagiosum
	Multiple painful vesicular, coalescing, recurring	Herpes simplex virus
	Nontender, indurated ulcer healing spontaneously 2–10 weeks	*Treponema pallidum*
	Non-indurated, painful papule, suppurative with adenopathy, slow to heal	*Haemophilus ducreyi*
	Soft, painless ulcer, pt from Caribbean or New Guinea, gram negative intracellular bacilli	*Klebsiella granulomatis (granuloma inguinale)*
Genital elephantiasis	Initial papule heals; lymph nodes enlarge and develop fistulas; genital elephantiasis may develop Tropics, microfilariae in bloodstream	*Chlamydia trachomatis* L1–L3 *Wuchereria* or *Brugia* (filarial nematodes)

Diarrhea

Dysentery

- Abdominal cramps, tenesmus, and pus and blood in the stool
- Usually associated with invasive bacterial disease in the colon

Diarrhea

- Refers to profuse watery feces
- Most commonly associated with increased secretion of fluid across the mucosal surfaces of the small intestine in response to a toxin or a viral infection
- No inflammatory cells, usually no fever

Table II-7-7. Diarrhea by Intoxication

Most Common Sources	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Ham, potato salad, cream pastries	All	**1–6 hours**	**Heat stable enterotoxin is produced in food contaminated** by food handler; food sits at room temperature	**abd cramps, vomiting, diarrhea; sweating and headache may occur; no fever**	<24 hours	***Staphylococcus aureus***
Rice	All	<6 hours	**Heat stable toxin causes vomiting**	As above	8–10 hours	***Bacillus cereus:* emetic form**
Meat, vegetables	All	>6 hours	Heat labile toxin causes diarrhea (similar to *E. coli* LT)	Nausea, abd cramps, diarrhea	20–36 hrs	*Bacillus cereus:* diarrheal form

Table II-7-8. Microbial Diarrhea: Organisms Causing Noninflammatory Diarrhea

Common Age Group Infected	Most Common Sources	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Infants and toddlers	Day care, water, nosocomial, fecal-oral	1–3 days (fall, winter, spring)	Microvilli of small intestine blunted; mononuclear infiltrate in lamina propria; disaccharidase activity down; glucose coupled transport normal; lactose intolerance may cause build up and osmotic influx creating watery diarrhea	Noninflammatory watery diarrhea, vomiting, fever, and dehydration	**5–7 days**	**Rotaviruses**
Young kids, IC	Nosocomial	7–8 days	?	Diarrhea, fever, and vomiting	8–12 days	**Adenovirus 40/41**
Infants in developing countries	Food, water, fecal-oral	2–6 days	**Adherence to enterocytes through pili causes damage to** adjoining microvilli	Watery to profusely watery diarrhea	1–3 weeks	**Enteropathogenic *E. coli***
Older kids and adults	Water, food, fecal-oral	18–48 hours	Jejunal biopsy shows blunting of microvilli; cytoplasmic vacuolization is seen along with mononuclear infiltrates of tissue; virus appears to decrease brush border enzymes causing malabsorption	Diarrhea, nausea, and vomiting; fever in some	12–48 hours	**Norwalk virus**
	Cruise ships					Noro-like virus
All	**Beef, poultry, gravies** Mexican food	8–24 hours	**Enterotoxin**	Abd cramps and **watery diarrhea**, rarely fever or vomiting	‹24 hours	***Clostridium perfringens***
All	Water, food, fecal-oral	9–72 hours	**Toxin stimulates adenylate cyclase and causes increase in cAMP in the small intestine without inflammation or invasion**	**Profuse watery diarrhea with vomiting; fever may be present** (rice water stools)	3–4 days	***Vibrio cholerae***
All	Raw or **undercooked shellfish**	5–92 hours	Self-limited gastroenteritis mimicking cholera; there is a severe, rarer dysentery form, no clear enterotoxin; hemolysins, phospholipase and lysophospholipase; tests for invasiveness are negative	Explosive watery diarrhea along with headache, abdominal cramps, nausea, vomiting, and fever.	Up to 10 days	***Vibrio parahaemolyticus***
All	Water, uncooked fruits and vegetables	12–72 hours	**Heat labile toxin (LT) stimulates adenylate cyclase resulting in efflux of water and ions into the small intestine**; stable toxin guanylate cyclase	Watery diarrhea with some vomiting and sometimes fever	3–5 days	**Enterotoxigenic *E. coli***
50% ‹10 yrs., all	Food, fecal-oral **(hamburger)**	3–5 days	**Verotoxin, which is a cytotoxin, causes bloody diarrhea** with no invasion of the organism	Abdominal cramps, watery diarrhea with blood **(no fever)**	7–10 days	**Enterohemorrhagic *E. coli.***
All	Water, day care, **camping,** beavers, dogs, etc.	5–25 days	Cysts ingested; excyst in the duodenum and jejunum; **multiply and attach to intestinal villi by sucking disk**	**Loose, pale, greasy diarrhea; mild to severe malabsorption** syndrome	1–2 weeks to years	***Giardia lamblia***
Children, AIDS patients	Day care, fecal-oral, animals, **homosexuals**	2–4 weeks	Sporozoites attach to the epithelial surface of the intestine and replicate	Mild diarrhea in immunocompetent; severe chronic diarrhea in AIDS **Acid-fast** spores/oocysts in stool	4 days to 3 weeks **in AIDS: indefinite**	***Cryptosporidium parvum*** ***Isospora belli, Cyclospora, Microsporidia***

Table II-7-9. Microbial Diarrhea: Organisms Causing Inflammatory Diarrhea/Dysentery

Common Age Group Infected	Most Common Sources*	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
All, esp <1 year and young adults	**Poultry, domestic animals, water,** unpasteurized milk, day care, fecal-oral	3–5 days	**Multiply in the small intestine; invades epithelium** resulting in inflammation and RBC and WBC in stools.	Diarrhea, abd pain, malaise; enteritis with diarrhea, malaise, fever	1–2 days mild; <1 week normal self-limiting	***Campylobacter jejuni***
All, esp infants and kids	**Poultry, domestic animals,** water, day care, fecal-oral	8–48 hours	Adsorb to epithelial cells in terminal small intestine; penetrate to lamina propria of ileocecal region causing **PMN response and PG response, which stimulates** cAMP and watery diarrhea	Diarrhea (occ bloody), abd cramps, abd tenderness, fever, and nausea w/occ vomiting; osteomyelitis in sickle cell anemia	3–5 days; spontaneous resolution	***Salmonella*** gastroenteritis
All, esp 6 mo to 10 yr.	Water, day care, no animal reservoirs, fecal-oral	1–7 days	*Shigella* colonize the small intestine producing at first an enterotoxin-induced watery diarrhea; ultimately the *shigellae* penetrate the colon mucosa producing **shallow mucosal ulcerations and dysentery; septicemia rare**	Watery diarrhea at first → lower abdominal cramps, tenesmus and abundant pus and blood in the stools (dysentery)	4–7 days; antibiotics can reduce spread	***Shigella***
All, esp older kids and young adults	Milk, wild **domestic animals** water, fecal-oral	2–7 days	The terminal ileum is infected with enlargement of the mesenteric lymph nodes; produces focal necrosis difficult to distinguish from appendicitis; organism is able to grow in cold; produces heat insensitive enterotoxin. Arthritis may occur.	Fever, diarrhea (frequently with leukocytes & blood in stools), abdominal pain; also a noninflammatory gastroenteritis	1 day – 3 weeks (avg. 9 days)	***Yersinia enterocolitica***
Pt on antibiotics	Associated with **antibiotic use (most common clindamycin)**	NA	Intense inflammatory response creates the friable yellow plaque-like colonic lesions (pseudo-membrane) associated with this disease	Mild diarrhea to severe **colitis;** abd cramps; spiking fever, systemic toxicity; blood, mucus, and pus in stools	Until antibiotic stopped; treat with metronidazole or change antibiotic	***Clostridium difficile***
Adults	Food, water, fecal-oral	2–3 days	Similar to *Shigella* dysentery	Fever and cramps with blood and pus in the stools	1–2 weeks; fluid and electrolyte replacement	**Enteroinvasive *E. coli***
All	Food, water, fecal-oral, **tropical** generally	2–4 weeks	Ingested cysts survive (trophozoites die) and multiply in the colon with invasion of the colon wall producing the **characteristic flask-like lesions and extra-intestinal abscesses**	Gen. acute diarrhea with cramping; sometimes dysentery; ulceration of colon may produce peritonitis	Weeks to months Rx with metronidazole followed by iodoquinol	***Entamoeba histolytica***

*Sources: Water = those listed are the most common diarrhea diseases spread through water

Day care = organisms listed are ones that have caused outbreaks in day care facilities, but note that any organism spread by the oral-fecal route may be a problem in this setting

Milk = unpasteurized milk or dairy products

Table II-7-10. Other Gastrointestinal or Liver Infections

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causal Agents
Hepatitis: Jaundice, anorexia, nausea, right upper quadrant pain on palpation, cigarettes taste foul, elevated liver enzymes*	Food-borne (possibly contaminated raw oysters or clams); 14–45 days; without chronicity; sturdy naked RNA virus	Hepatitis A ("infectious" hepatitis) (picornavirus)
	IV drug abuse, needle stick; chronic carrier state, cirrhosis, primary hepatocellular carcinoma; DNA virus easily inactivated by alcohol	Hepatitis B ("serum" hepatitis) neonatal transmission (Hepadnavirus)
	Transfusion, IV drug abuse, or prison-acquired tattoos; acute illness is less severe than hepatitis B but chronicity is higher, with 60% of those infected having chronic active hepatitis; RNA, enveloped virus	Hepatitis C (Flavivirus)
	Enterically transmitted with high fatality in pregnant women, no chronic form	Hepatitis E (Hepevirus)
Acute abdominal pain	Intestinal blockage	*Ascaris lumbricoides or Diphyllobothrium latum*
Bile duct blockage		*Ascaris lumbricoides* (following surgery) *Fasciola hepatica*
Peritonitis		Mixed flora often involving anaerobic normal flora: *Bacteroides fragilis* and facultative anaerobes such as *E. coli*
Cirrhosis	Travel history: Puerto Rico, Peace Corps, etc.; egg granulomas block triads → fibrosis	*Schistosoma mansoni*
	IV drug use	Hepatitis viruses
Pancreatitis	Generally with swelling of salivary glands	Mumps virus

*Hepatitis may also occur with two other viruses: CMV and yellow fever virus or with toxoplasmosis or leptospirosis.

Table II-7-11. Changes in Blood Cells

Symptoms and Signs	Case Vignette/Key Clues	Most Common Causal Agents
Anemia	Megaloblastic	*Diphyllobothrium latum*
	Normocytic	Chronic infections
	Microcytic and hypochromic (iron deficiency anemia)	*Ancylostoma, Necator, Trichuris*
Patient with cyclic or irregular fever, decreased hemoglobin and hematocrit	Often foreign travel to tropics, rings or schizonts in RBCs	*Plasmodium*
Splenectomized patient, New England, hemolytic anemia, no travel history, summer months (tick exposure)	Multiple ring forms inside RBC	*Babesia microti*
Reduced CD4 cell count		HIV
Increases in PMNs		Generally found in many extracellular bacterial infections
Increases in eosinophils		Allergy
		Helminths during migrations
Increases in mononuclear leukocytes (monocytes or lymphocytes)		Viruses and other intracellular organisms: *Listeria, Legionella, Leishmania, Toxoplasma*
	Infectious mononucleosis Heterophile (+)Downey type II cells (reactive T cells) sore throat, lymphadenopathy, young adult	Epstein-Barr virus (EBV)
	Heterophile negative	CMV *Toxoplasma* *Listeria* (Listeriosis)
Lymphocytosis with paroxysmal cough, stridor on inspiration	Unvaccinated child, hypoglycemic	*Bordetella pertussis*

Table II-7-12. Central Nervous System Infections

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causal Agents
Meningitis: Headache, fever, vomiting, sepsis, seizures, irritability, lethargy, bulging fontanelles, nuchal rigidity	Neonate to 2 months	*Streptococcus agalactiae* #1 (gram-positive coccus) *E. coli* (gram-negative rod) More rarely: *Listeria monocytogenes* (motile gram-positive rod)
	3 months to 2 years; unvaccinated child	*Haemophilus influenzae* type B* (gram-negative pleomorphic rod with polyribitol capsule)
	3 mo to young adult. Prodrome may be very rapid; child may be properly vaccinated; rash	*Neisseria meningitidis* (gram-negative diplococcus with capsule; ferments maltose)
	<2 yrs Young adults to elderly	*Streptococcus pneumoniae* (gram-positive coccus, catalase negative, alpha hemolytic, inhibited by optochin, lysed by bile
	Renal transplant patient	*Cryptococcus neoformans* (#1); encapsulated, urease (+) yeast *Listeria monocytogenes* (motile gram-positive rod)
	Several month prodrome (except in severely compromised). Usually some underlying condition, endemic area	Fungal, e.g., Cryptococcal, or if in Southwestern U.S., *Coccidioides* If near U.S. great river beds with exposure to bird, bat feces; *Histoplasma capsulatum*
	Patient with low CMI, nerve palsies in a patient with tuberculosis and low CSF glucose	*Mycobacterium tuberculosis* (Tuberculous meningitis)
Meningoencephalitis:	Swimming and often diving in very warm waters (hot springs). Prefrontal headache, high fever, disturbance of smell	*Naegleria*
	Immunocompromised patients	*Acanthamoeba* or *Toxoplasma*

*By 1990, with day care centers and the dramatic increase in *Haemophilus* meningitis, *Haemophilus* meningitis became overall the most common. Since late 1990, when the conjugated vaccine went into use, there has been a dramatic decrease in *Haemophilus* meningitis in **vaccinated kids**.

(*Continued*)

Table II-7-12. Central Nervous System Infections (*continued*)

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causal Agents
Encephalitis: Headache, and fever → drowsiness, coma, hemiplegia, cranial nerve palsy, hallucinations, behavioral disturbances, and other focal neurological findings	Summer–fall, mosquito-borne from bird reservoirs (except for California encephalitis, which is a rodent reservoir)	Encephalitis with **arboviruses**: **Western equine encephalitis** (midwest and west U.S.) **St. Louis encephalitis** elderly blacks with hypertension, most severe infections **West Nile Virus** (North America) **California encephalitis** (entire U.S.) **Eastern equine encephalitis** all age groups, but most common in young and old; highest morbidity of viral CNS infections; with mental retardation, seizures, personality changes in survivors
	Focal uptake of radionucleotide, RBCs in CSF, high opening pressure, frontal temporal lobe involvement.	**Herpes simplex encephalitis**
Mass lesion	Generally following: sinus, ear, or dental infection, infection at distant site, head trauma, etc. (symptoms dependent on location of mass) and elevated intracranial pressure along with headache, mental changes, nausea, vomiting, fever with chills, and seizure	Don't do lumbar puncture; CT generally shows ring enhancing lesion; 45% mixed infections; Streptococci and *Bacteroides* are the two most commonly identified groups of bacteria
Reye Syndrome	Child following a viral illness with pernicious vomiting, lethargy and irritability, which may lead to brain swelling, indication of aspirin usage.	Influenza or varicella infection (Reye syndrome)
Epilepsy	Mexico, immigrant, onset after age 20	*Taenia solium* (neurocysticercosis)
Bell palsy (acute facial nerve paralysis)	Systemic disease following bull's-eye rash	*Borrelia burgdorferi*
Guillain-Barré (acute inflammatory demyelinating polyneuropathy with ascending paralysis)	With GI tract problems	*Campylobacter jejuni*
	With respiratory problems	*Influenza*

Table II-7-13. Cerebrospinal Fluid Finding in Meningitis

Pressure	CSF Appearance	Cell Count (cells/mm)^3	Dominant Cell Type	Glucose mg/dL	Protein mg/dL	Condition
<100 mm H2O	Clear	0–5	Lymphocytes	40–70	<40	Normal
Normal or +	Clear	0–500	Early: PMNs Late: lymphocytes	Normal or –	Normal or +	Viral infection
++	Opaque	1–60,000	PMNs	–	++	Bacterial infection
+	Clear	10–500	Early: PMNs Late: lymphocytes	–	+ to ++	Fungal infection

– Below normal range, + above normal range

Table II-7-14. Selected Rashes

Type Rash	Progression	Other Symptoms	Disease	Causal Agent/Toxin
Erythematous maculopapular rash (sandpaper-like rash)	Trunk and neck → extremities	Sore throat, fever, nausea	Scarlet fever	*Strep. pyogenes* Exotoxin A-C
Diffuse erythematous, macular, sunburn-like rash	Trunk and neck → extremities with desquamation on palms and soles	Acute onset, fever >38.8 C (102.0 F), myalgia, pharyngitis, vomiting, diarrhea; hypotension leading to multi-organ failure	Toxic shock syndrome	*Staph. aureus* TSST-1
Perioral erythema, bullae, vesicles, desquamation	Trunk and neck → extremities, except tongue and palate; large bullae and vesicles precede defoliation	Abscess or some site of infection	Staphylococcal skin disease: scalded skin disease & scarlatina	*Staph. aureus* Exfoliatin
Petechiae → purpura	Trunk → extremities; spares palms, soles, and face	Fever, rash, headache, myalgias, and respiratory symptoms	Epidemic typhus	*Rickettsia prowazekii* ? Endotoxin
	Ankles and wrists → generalized with palms and soles	Fever, rash, headache, myalgias, and respiratory symptoms	Rocky Mountain spotted fever (most common on East Coast)	*Rickettsia rickettsii* ? Endotoxin
	Generalized	Abrupt onset, fever, chills, malaise, prostration, exanthem → shock	Early meningococcemia	*N. meningitidis* Endotoxin
Skin: maculopapular; mucous membrane: condyloma	Generalized involving the palms and soles, bronze or copper colored	Fever, lymphadenopathy, malaise, sore throat, splenomegaly, headache, arthralgias	Secondary syphilis	*Trep. pallidum* Endotoxin
Confluent erythematous maculopapular rash	Head → entire body	Cough coryza, conjunctivitis, and fever (prodrome), oral lesions (Koplik spots), exanthem, bronchopneumonia, and ear infections	Measles	Rubeola virus Rash from T cell destruction of virus-infected cells in capillaries
Erythematous concentric rings (Bull's eye)	Outward from site of tick bite	Fever, headaches, myalgias, Bell's palsy	Lyme disease	*Borrelia burgdorferi*

Table II-7-15. Osteomyelitis

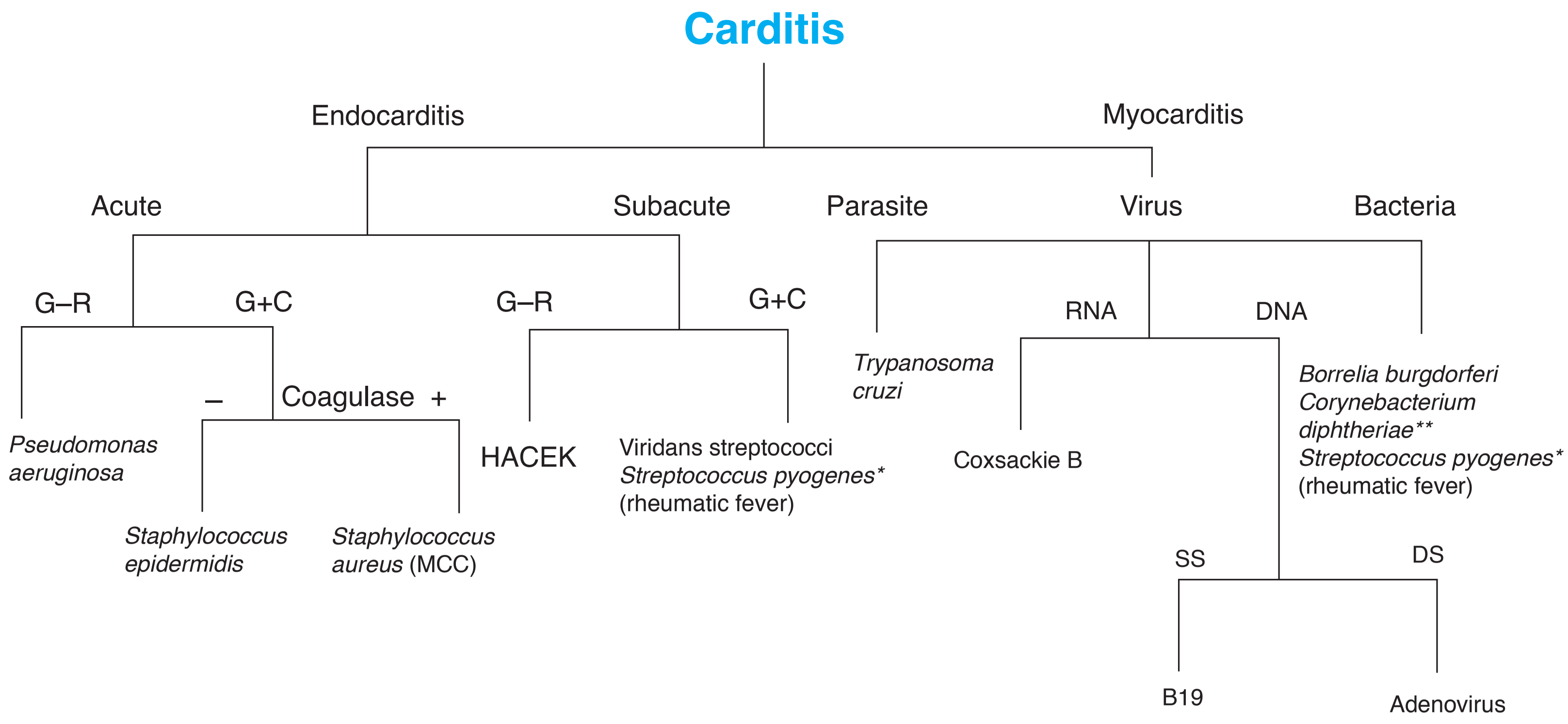
Type Infection	Case Vignette/Key Clues	Most Common Causal Agents
Fever, bone pain with erythema and swelling, some patients (diabetic particularly) may have associated cellulitis	Adults, children, and infants without major trauma or special conditions	*Staphylococcus aureus*
	Neonates (‹1 mo)	*Staphylococcus aureus* Group B *Streptococcus*, Gram-negative rods (*E. coli, Klebsiella, Proteus, Pseudomonas*)
	Sickle cell anemia*	*Salmonella*
	Trauma	*Pseudomonas*

* Sickle cell anemia patients are functionally asplenic and may have defective opsonic and alternate complement pathway activities. The most common bacterial infections include

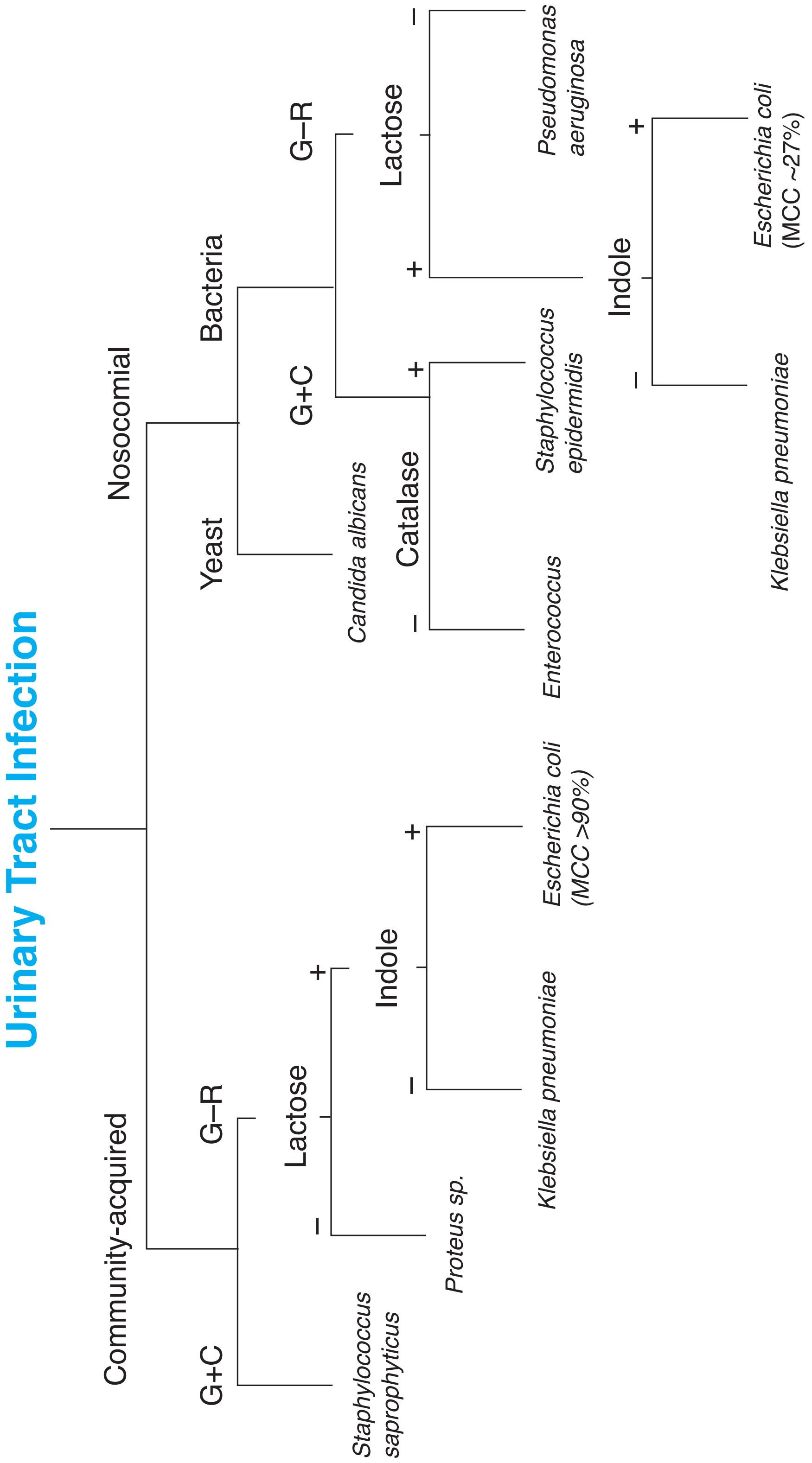
- Encapsulated organisms
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Neisseria meningitidis*
  - *Salmonella enterica* subsp.
- Osteomyelitis due to *Salmonella enterica* subsp.
- Pneumonia, bacteremia, and meningitis are all a problem.

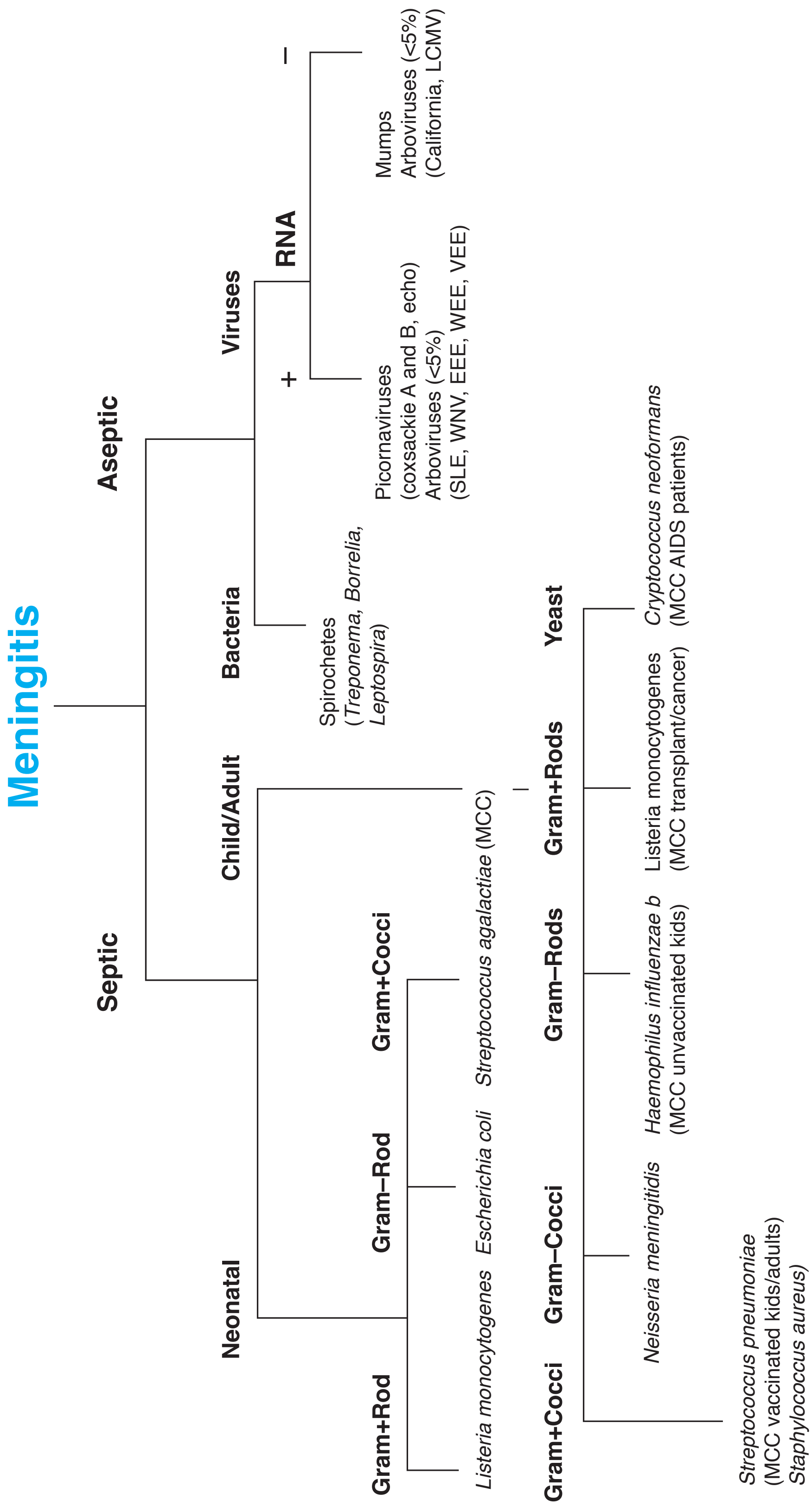
Table II-7-16. Arthritis Related to Infections

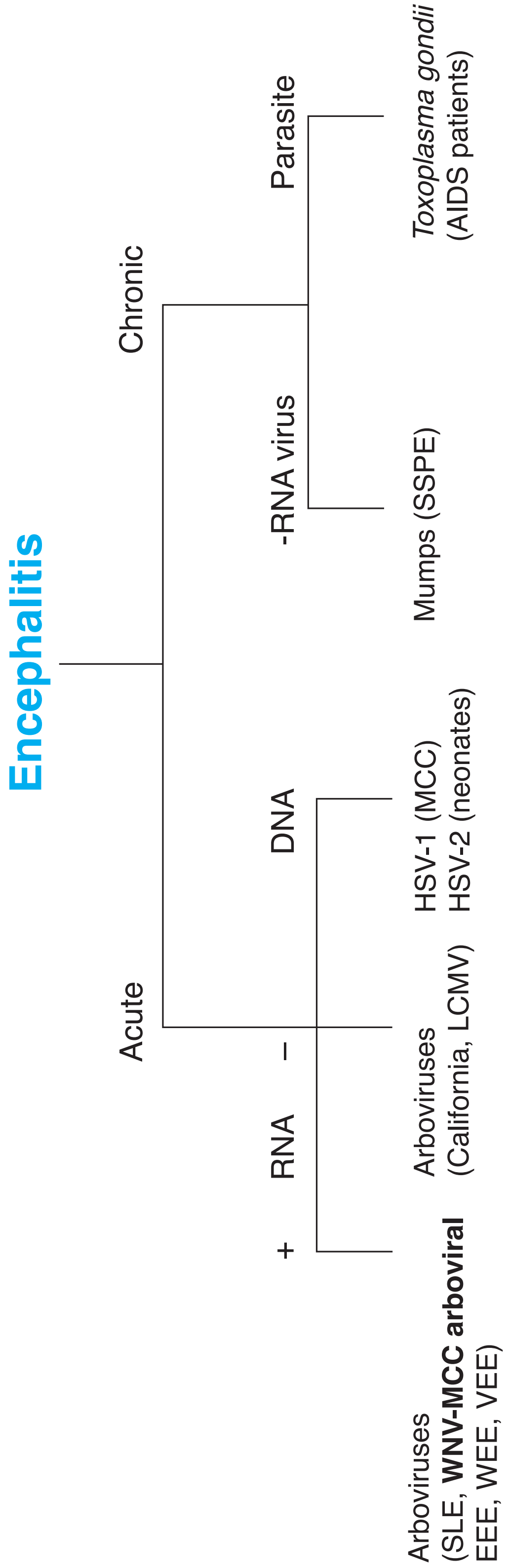
Type Infection	Case Vignette/Key Clues	Most Common Causal Agents
Pain, redness, low-grade fever, tenderness, swelling, reduced joint mobility	#1 overall except in the 15–40 age group where gonococcal is more prevalent	*Staphylococcus aureus*
	Multiple joints	From septicemia, e.g., staphylococci, gonococci
	15–40 years; mono- or polyarticular	*Neisseria gonorrhoeae*
	Prosthetic joint	Coagulase negative staphylococci
	Viral	Rubella, hepatitis B, and parvovirus
	Chronic onset, monoarticular	*M. tuberculosis* or fungal
	Large joint resembling Reiter following tick bite or erythema migrans	*Borrelia burgdorferi*
Postinfectious (reactive arthritis)	Following gastrointestinal infection	*Salmonella, Shigella, Campylobacter,* or *Yersinia enterocolitica*
	Following sexual contact	*Chlamydia trachomatis*

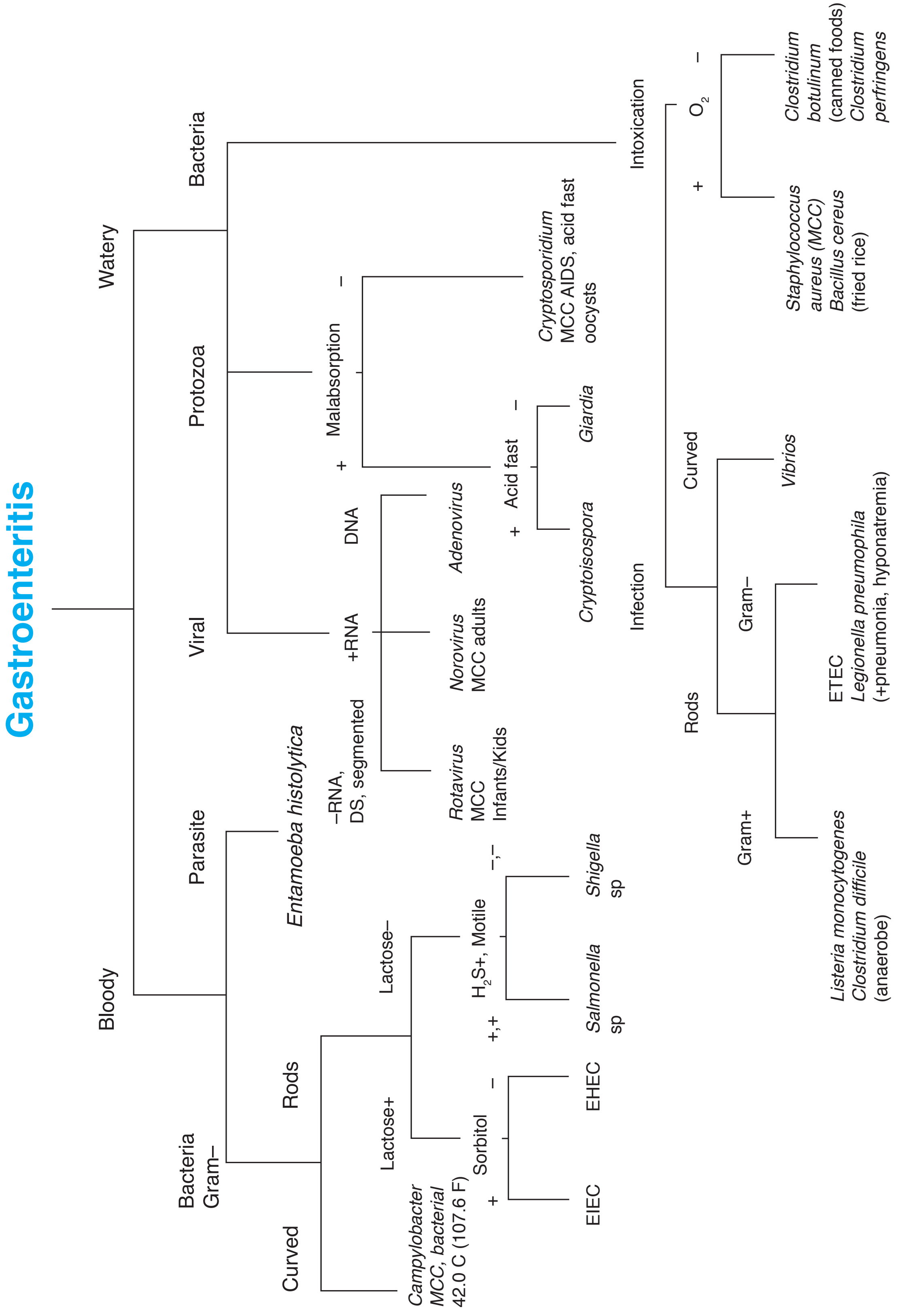


*Rheumatic fever can also be classified as pancarditis, so it is listed under both endocarditis and myocarditis.
**Corynebacterium diphtheriae is a local infection, carditis, due to systemic effects of the toxin.

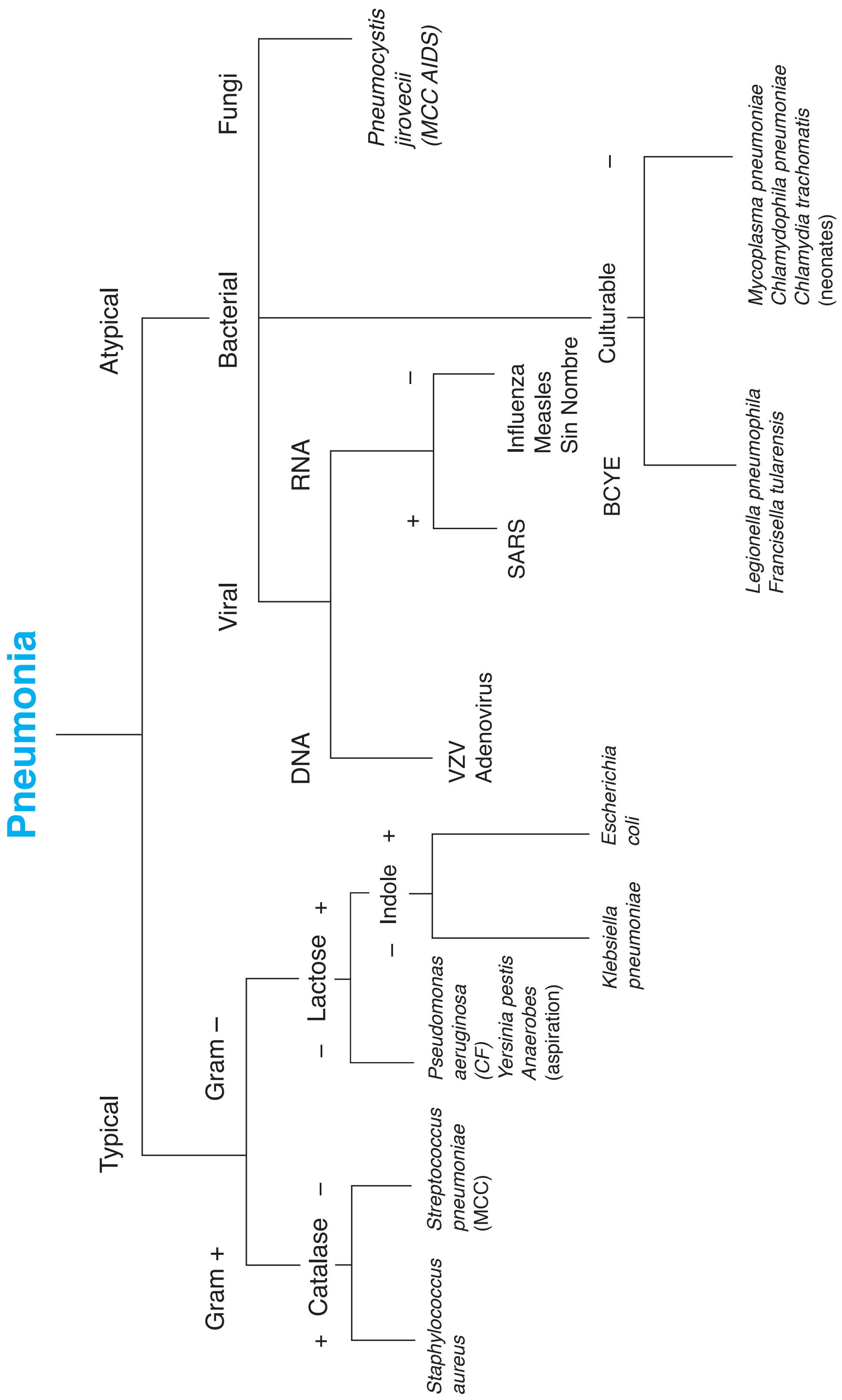

Urinary Tract Infection
Community-acquired
G+C
Staphylococcus saprophyticus
G–R
Lactose
–
Proteus sp.
+
Indole
–
Klebsiella pneumoniae
+
Escherichia coli (MCC >90%)
Nosocomial
Yeast
Candida albicans
Bacteria
G+C
Catalase
–
Enterococcus
+
Staphylococcus epidermidis
G–R
Lactose
–
Pseudomonas aeruginosa
+
Indole
–
Klebsiella pneumoniae
+
Escherichia coli (MCC ~27%)


Meningitis
Septic
Aseptic
Neonatal
Child/Adult
Bacteria
Viruses
Gram+Rod
Gram−Rod
Gram+Cocci
Listeria monocytogenes
Escherichia coli
Streptococcus agalactiae (MCC)
Gram+Cocci
Gram−Cocci
Gram−Rods
Gram+Rods
Yeast
Streptococcus pneumoniae
(MCC vaccinated kids/adults)
Staphylococcus aureus)
Neisseria meningitidis
Haemophilus influenzae b
(MCC unvaccinated kids)
Listeria monocytogenes
(MCC transplant/cancer)
Cryptococcus neoformans
(MCC AIDS patients)
Spirochetes
(Treponema, Borrelia,
Leptospira)
RNA
+
−
Picornaviruses
(coxsackie A and B, echo)
Arboviruses (<5%)
(SLE, WNV, EEE, WEE, VEE)
Mumps
Arboviruses (<5%)
(California, LCMV)

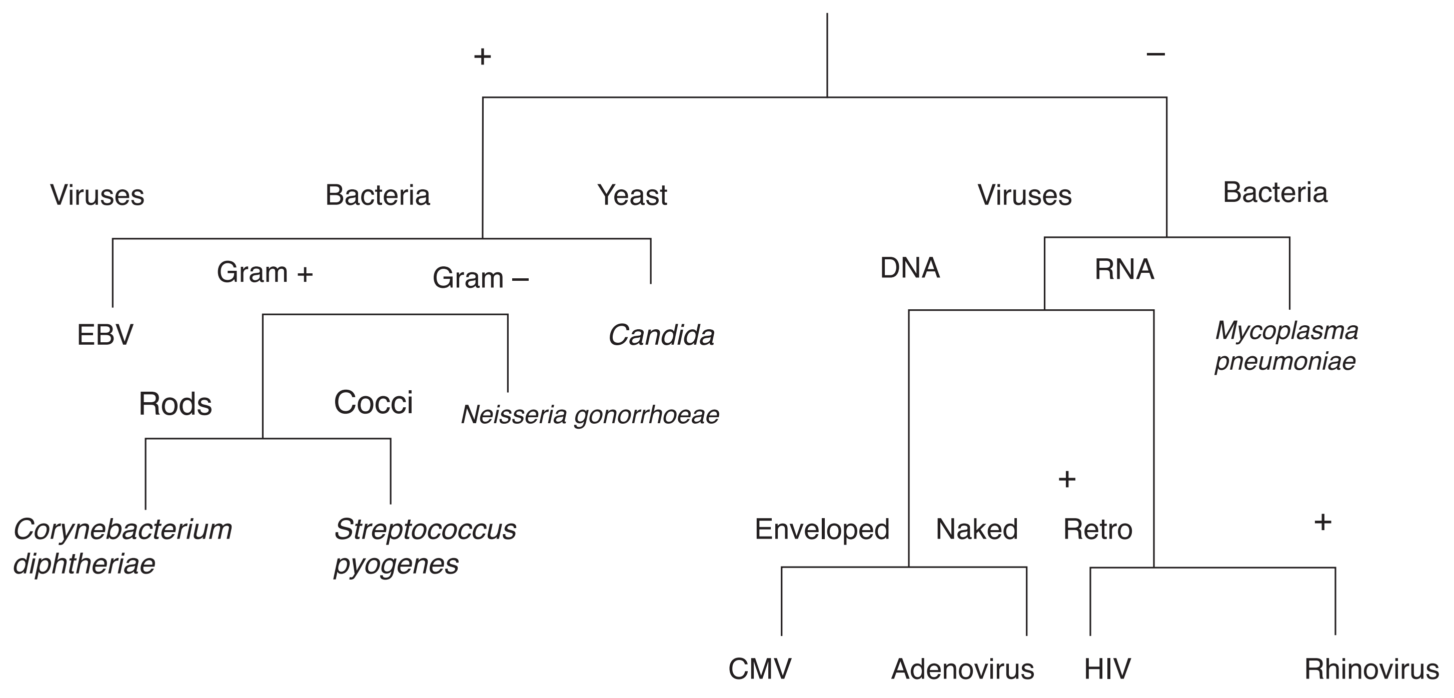

Encephalitis
Acute
Chronic
RNA
+
–
DNA
-RNA virus
Parasite
Arboviruses (SLE, **WNV-MCC arboviral** EEE, WEE, VEE)
Arboviruses (California, LCMV)
HSV-1 (MCC)
HSV-2 (neonates)
Mumps (SSPE)
Toxoplasma gondii (AIDS patients)



Gastroenteritis
Bloody
Watery
Bacteria Gram–
Parasite
Viral
Protozoa
Bacteria
Curved
Rods
Entamoeba histolytica
Campylobacter
MCC, bacterial
42.0 C (107.6 F)
Lactose+
Lactose–
+
Sorbitol
–
EIEC
EHEC
+,+
H_2S+, Motile
–,–
Salmonella sp
Shigella sp
–RNA,
DS, segmented
+RNA
DNA
Rotavirus
MCC
Infants/Kids
Norovirus
MCC adults
Adenovirus
+
Malabsorption
–
+
Acid fast
–
Cryptoisospora
Giardia
Cryptosporidium
MCC AIDS, acid fast
oocysts
Infection
Intoxication
Rods
Curved
Gram+
Gram–
Vibrios
Listeria monocytogenes
Clostridium difficile
(anaerobe)
ETEC
Legionella pneumophila
(+pneumonia, hyponatremia)
+
O_2
–
Staphylococcus
aureus (MCC)
Bacillus cereus
(fried rice)
Clostridium
botulinum
(canned foods)
Clostridium
perfringens


Pneumonia
Typical
Atypical
Gram +
Gram –
Viral
Bacterial
Fungi
+ Catalase –
– Lactose +
DNA
RNA
Staphylococcus aureus
Streptococcus pneumoniae (MCC)
Pseudomonas aeruginosa (CF)
Yersinia pestis
Anaerobes (aspiration)
– Indole +
Klebsiella pneumoniae
Escherichia coli
VZV
Adenovirus
+
–
SARS
Influenza
Measles
Sin Nombre
Pneumocystis jirovecii (MCC AIDS)
BCYE
Culturable
–
Legionella pneumophila
Francisella tularensis
Mycoplasma pneumoniae
Chlamydophila pneumoniae
Chlamydia trachomatis (neonates)

Pharyngitis*



*Viruses, in general, are the most common cause of pharyngitis. The above listed viruses are most common and most relevant for the exam.

Comparative Microbiology 8

Learning Objectives

- ❑ Differentiate infectious organisms that have clinically relevant and distinctive morphology, physiology, pathogenicity, epidemiology, transmission, pathology, lab diagnosis, treatment, or prevention

MORPHOLOGY/TAXONOMY

Spore-Forming Bacteria (Have Calcium Dipicolinate)

Bacillus
Clostridium

Non-motile Gram-Positive Rods

Corynebacterium diphtheriae
Nocardia
Clostridium perfringens (rest of the pathogenic *Clostridia* are motile)
Bacillus anthracis (most other *Bacillus* species are motile)

Acid Fast Organisms

Mycobacterium
Nocardia (partially acid fast)
***Cryptosporidium* oocysts**
Isospora oocysts

Bacteria and Fungi That Characteristically Have Capsules

The "biggies" can be remembered by the mnemonic: **S**ome **K**illers **H**ave **P**retty **N**ice **C**apsules!

Streptococcus pneumoniae
Klebsiella pneumoniae
Haemophilus influenzae
Pseudomonas aeruginosa—slime producer especially in cystic fibrosis patients' lungs
Neisseria meningitidis
Cryptococcus neoformans (only encapsulated fungal pathogen)
Bordetella pertussis

Other Important Capsule Producers

E. coli meningeal strains have capsule, mostly K_1

Bacillus anthracis—poly D-glutamate capsule

Salmonella enterica subsp. *typhi*—(virulence; Vi) capsular antigen

Streptococcus pyogenes when first isolated; non-immunogenic (but anti-phagocytic) hyaluronic acid capsule

Biofilm Producers

Staphylococcus epidermidis (catheter-related infections)

Streptococcus mutans (dental plaque)

Pigment Production

***Pseudomonas aeruginosa* (blue-green)—pyocyanin, fluorescein**

Serratia—red pigment

Staphylococcus aureus—yellow pigment

Photochromogenic and scotochromogenic *Mycobacteria*—Carotenoid pigments (yellow and orange)

Corynebacterium diphtheriae—black to gray

Unique Morphology/Staining

Metachromatic staining—*Corynebacterium*

Lancet-shaped diplococci—*Pneumococcus*

Kidney bean-shaped diplococci—*Neisseriae*

Bipolar staining—***Yersinia pestis***

Gulls wings—*Campylobacter*

Table II-8-1. Viral Cytopathogenesis

Inclusion Bodies	Virus
Intracytoplasmic (Negri bodies)	Rabies
Intracytoplasmic acidophilic (Guarnieri)	Poxviruses
Intranuclear (Owl eye)	Cytomegalovirus
Intranuclear (Cowdry)	Herpes simplex virus Subacute sclerosing panencephalitis (measles) virus
Syncytia formation	**Virus** Herpes viruses Varicella-zoster Paramyxoviruses (measles, mumps, rubella and respiratory syncytial virus) HIV

PHYSIOLOGY

Table II-8-2. Metabolism*

Aerobes	Anaerobes	Microaerophilic
Mycobacterium	***Actinomyces***	***Campylobacter***
Pseudomonas	***Bacteroides***	***Helicobacter***
Bacillus	***Clostridium***	
Nocardia	*Fusobacterium*	
Corynebacterium diphtheriae	*Prevotella*	
	Propionibacterium (aerotolerant)	
	Eubacterium	
	Lactobacillus (aerotolerant)	

*Most others are considered facultative anaerobes.

Enzymes

Oxidase

- **All Enterobacteriaceae are oxidase negative.**
- **All *Neisseria* are oxidase positive** (as are most other Gram-negative bacteria).

Urease Positive (mnemonic: PUNCH)

- All ***Proteus* species** produce urease; this leads to alkaline urine and may be associated with renal calculi.
- *Ureaplasma* (renal calculi)
- *Nocardia*
- *Cryptococcus* (the fungus)
- ***Helicobacter***

Catalase

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2$$

***Staphylococci* have catalase, *Streptococci* do not.**

Most anaerobes lack catalase.

Catalase positive organisms are major problems in chronic granulomatous disease (CGD):

- All staphylococci
- *Pseudomonas aeruginosa*
- *Candida*
- *Aspergillus*
- Enterobacteriaceae

Coagulase positive

- ***Staph. aureus***
- ***Yersinia pestis***

DETERMINANTS OF PATHOGENICITY

Genetics

Genes encoding pathogenic factors reside on:

- The bacterial **chromosome**

  Endotoxin

- A **plasmid**

  Most toxins and multiple drug resistances

- A **bacteriophage genome** stably integrated into the host DNA as a prophage. Virulence modified by the stable presence of phage DNA in bacterial cell = lysogenic conversion.

  Examples:

  C = cholera toxin

  O = *Salmonella* O antigen

  B = Botulinum toxin (phage CEβ and DEβ)

  E = Erythrogenic toxin of *Streptococcus pyogenes*

  D = Diphtheria toxin (Corynephage β)

  S = Shiga toxin

  Mnemonic: **COBEDS** (when 2 people share a bed somebody gets a little pregnant [with phage])

Antigenic variation

Neisseria gonorrhoeae (pili)

Borrelia recurrentis

Trypanosoma brucei

HIV

Toxins

Table II-8-3. Disease Due to Toxin Production

Bacterium	Disease	Activity of Toxin
Corynebacterium diphtheriae	**Diphtheria**	ADP ribosylation of eEF-2 results in inhibition of protein synthesis
Clostridium tetani	**Tetanus**	Binds to SV2 ganglioside protein receptor. SV2 enables internalization and cellular intoxication.
Clostridium botulinum	**Botulism**	Prevents release of acetylcholine
Vibrio cholerae	**Cholera**	Choleragen stimulates adenylate cyclase
E. coli (ETEC)	**Traveler's diarrhea**	LT stimulates adenylate cyclase
Clostridium difficile	Diarrhea	Toxin A and B inhibit protein synthesis and cause loss of intracellular K^+
Bordetella pertussis	Whooping cough	Hypoglycemia due to activation of islets Edema due to inhibition Gi Lymphocytosis due to inhibition of chemokine receptors Sensitivity to histamine

eEF-2 = eukaryotic elongation factor-2

Heat stable toxins

60.0 C (140.0 F)

- *Staphylococcus aureus* enterotoxin
- ST toxin of *E. coli*
- *Yersinia enterocolitica* toxin

100.0 C (212.0 F)

- Endotoxin

Toxins with ADP-ribosylating activity

Table II-8-4. Toxins with A-B ADP-Ribosyl Transferase Activity

Toxin	ADP-Ribosylated Host Protein	Effect on Host Cell
Pseudomonas Exotoxin A Exotoxin S	 eEF-2 unknown	Inhibits translocation during protein synthesis
Diphtheria toxin	eEF-2	Inhibits translocation during protein synthesis
E. coli heat-labile toxin (LT)	G-protein (G_S)	Increases cAMP in intestinal epithelium causing diarrhea
Cholera toxin	G-protein (G_S)	Increases cAMP in intestinal epithelium causing diarrhea
Pertussis toxin	G-protein (G_i)	Increases cAMP causing edema, lymphocytosis and increased insulin secretion

A is the ADP-ribosyl transferase.
B binds to cell receptor and translocates the A subunit into the cell.

Invasive factors

Table II-8-5. Invasive Factors

Invasive Factor	Function	Bacteria
All capsules	Antiphagocytic	See earlier list with morphology
Slime layer (capsule or glycocalyx)	Antiphagocytic	*Pseudomonas*
M protein	Antiphagocytic	Group A Streptococci
A protein	Inhibits opsonization	*Staph. aureus*
Lipoteichoic acid	Attachment to host cells	All gram-positive bacteria
N. gonorrhoeae pili	Antiphagocytic	*N. gonorrhoeae*

Table II-8-6. Extracellular Enzymes

Enzyme	Function	Bacteria
Hyaluronidase	Hydrolysis of ground substance	Group A Streptococci
Collagenase	Hydrolysis of collagen	*Clostridium perfringens* *Prevotella melaninogenica*
Kinases	Hydrolysis of fibrin	*Streptococcus* *Staphylococcus*
Lecithinase (alpha toxin)	Damage to membrane	*Clostridium perfringens*
Heparinase	May contribute to thrombophlebitis	*Bacteroides fragilis* *Prevotella melaninogenica*
IgA Proteases	Colonizing factor	*Neisseria* *Haemophilus* *Strep. pneumoniae*

Ability to Survive and Grow in Host Cell

Obligate Intracellular Parasites

Cannot be cultured on inert media. Virulence is due to the ability to survive and grow intracellularly where the organism is protected from many B-cell host defenses.

- Bacteria

  All Rickettsiae

  All Chlamydiaceae

  Mycobacterium leprae

- Viruses

  All are obligate intracellular parasites.

- Protozoa

  Plasmodium

  Toxoplasma gondii

  Babesia

  Leishmania

  Trypanosoma cruzi (amastigotes in cardiac muscle)

- Fungi

  None

Facultative intracellular parasites of humans

- Bacteria
  - *Francisella tularensis*
  - *Listeria monocytogenes*
  - *Mycobacterium tuberculosis*
  - *Brucella* species
  - Non-tuberculous *mycobacteria*
  - *Salmonella enterica* subsp. *typhi*
  - *Legionella pneumophila*
  - *Yersinia pestis*
  - *Nocardia* species
- Fungi
  - *Histoplasma capsulatum*

Obligate Parasites That Are Not Intracellular

(e.g., cannot be cultured on inert media but are found extracellularly in the body)

- *Treponema pallidum*
- *Pneumocystis jirovecii*

EPIDEMIOLOGY/TRANSMISSION

Bacteria That Have Humans as the Only Known Reservoir

Mycobacterium tuberculosis

***M. leprae* (armadillos in Texas)**

***Shigella* species**

Salmonella enterica* subspecies *typhi

***Rickettsia prowazekii* (epidemic typhus)**

Group A β-hemolytic *Streptococcus*

Neisseria meningitidis and *N. gonorrhoeae*

Corynebacterium diphtheriae

Streptococcus pneumoniae

Treponema pallidum

Chlamydia trachomatis

Zoonotic Organisms

(Diseases of animals transmissible to humans)

Bacillus anthracis

Salmonella enterica all subspecies except *typhi*

Leptospira

Borrelia

Listeria monocytogenes
Brucella species
Francisella tularensis
Pasteurella multocida (cat bites)
Vibrio parahaemolyticus (from fish)
Capnocytophaga canimorsus (dog bites)
Bartonella henselae (cat scratches)
Streptobacillus moniliformis (rat bite fever)
Mycobacterium marinum (fish tank granuloma)
Vibrio vulnificus (oysters)
Yersinia pestis, Y. enterocolitica, Y. pseudotuberculosis
Campylobacter fetus, C. jejuni
Most Rickettsia
Chlamydophila psittaci (birds)
Rabies virus

Arthropod Vectors in Human Disease: Insects

- Lice
  - Epidemic or louse-borne typhus (*Pediculus h. humanus*)
  - Epidemic relapsing fever
  - Trench fever
- **True bugs**
  - **Chagas' disease (American trypanosomiasis)—kissing bugs (Reduviidae)**
- Mosquitoes
  - **Malaria (*Anopheles* mosquito)**
  - **Dengue (*Aedes*)**
  - **Mosquito-borne encephalitides: WEE, EEE, VEE, SLE, WNV**
  - **Yellow Fever (*Aedes*)**
  - Filariasis
- **Sandflies**
  - **Leishmaniasis**
  - Bartonellosis
- Midges
  - Filariasis
- Blackflies
  - Onchocerciasis
- Deerflies (*Chrysops*) and horse flies
  - Loiasis
  - Tularemia

- **Tsetse flies**

  African trypanosomiasis

- **Fleas**

  Plague

  Endemic typhus

Arthropod Vectors That Are Not Insects

- **Ticks**

  Rocky Mountain spotted fever (*Dermacentor*)

  Colorado tick fever (*Dermacentor*)

  Lyme disease (*Ixodes*)

  Ehrlichia (Ixodes, Amblyomma)

  Babesiosis (*Ixodes*)

  Tularemia (*Dermacentor*)

  Recurrent fever or tick-borne relapsing fever (*Ornithodoros*, a soft tick)

- Mites

  Scrub typhus (*Leptotrombidium*) (transovarial transmission in vector)

  Rickettsialpox

Parasitic Infections Transmitted by Ova

Enterobius vermicularis (pinworm)

Ascaris lumbricoides (roundworm)

Toxocara canis (visceral larva migrans)

Trichuris trichiura (whipworm)

Echinococcus granulosus/multilocularis

Taenia solium (cysticercosis)

All others are transmitted in larval stage.

Bacterial and Fungal Infections That Are Not Considered Contagious

(i.e., no human-to-human transmission)

Nontuberculous mycobacterial infections, e.g., ***Mycobacterium avium-intracellulare***

Non-spore forming anaerobes

Legionella pneumophila

All fungal infections except the dermatophytes

Infections That Cross the Placenta

(Mnemonic: TORCH)

T*oxoplasma*

Other (Syphilis)

Rubella

CMV

Herpes and **H**IV

<5% perinatal hepatitis B could possibly have been acquired by crossing placenta.

- Viruses
  - *Cytomegalovirus*
  - *Rubella*
  - HSV 2 (in primary infection)
  - Coxsackie B
  - Polio
  - HIV
  - B19
- Parasites
  - ***Toxoplasma gondii***
- Bacteria
  - ***Treponema pallidum***
  - ***Listeria monocytogenes***

Spread by Respiratory Droplet

Streptococcus pyogenes (Group A)

Streptococcus pneumoniae

Neisseria meningitidis

Mycobacterium tuberculosis

Bordetella pertussis

Haemophilus influenzae

Corynebacterium diphtheriae

Mycoplasma pneumoniae

Influenza

Rubella

Measles

Chickenpox

Pneumocystis jirovecii

Spread by Inhalation of Organisms from the Environment

Histoplasma

Coccidioides

Blastomyces

Nontuberculous mycobacteria, e.g., *M. avium-intracellulare* (*MAC*)

Legionella

Chlamydophila psittaci

Pseudomonas (also spread by ingestion and contact)

Spread by Oral/Fecal Route

(Infections may be spread by oral sex.)

Salmonella
Shigella
Campylobacter
Vibrio
Yersinia enterocolitica
Yersinia pseudotuberculosis
Bacillus cereus
Clostridium
Staphylococcus (also other routes commonly)

Enteroviruses, including poliovirus
Rotavirus
Norwalk agent
Hepatitis A
Toxoplasma—cat feces
Entamoeba
Giardia

All nematodes except filaria and *Trichinella*
All cestodes

Contact: (Person-to-Person) Nonsexual

Impetigo (*Strep* and *Staph*)
Staphylococcus
Herpes I
Epstein-Barr (kissing)
Hepatitis B (all body fluids)
Molluscum contagiosum (wrestling teams)

Contact: Sexual

Chlamydia	HPV	HBV
Neisseria	HIV	HCV
Treponema	HSV 2	
Trichomonas	CMV	

PATHOLOGY

Organisms that Produce Granulomas (most are intracellular, others have persistent antigen)

Fran **L**ikes **M**y **P**al **B**ruce **A**nd **H**is **B**lasted **C**ockerspaniel (in) **S**alt **L**ake **C**ity. (Mnemonic by M. Free.)

(ic) = intracellular organism

***F**rancisella* (ic)

***L**isteria* (ic)

***M**ycobacterium* (ic)

*Treponema **p**allidum*

***B**rucella* (ic)

***A**ctinomyces*

***H**istoplasma* (ic)

***B**lastomyces*

***C**occidioides*

***S**chistosoma* species

Lymphogranuloma venereum (ic)

Cat scratch fever

Infections Causing Intracerebral Calcifications

Toxoplasma

CMV

Cysticercosis

Cryptococcus neoformans

Tuberculous meningitis

LABORATORY DIAGNOSIS

Special Stains

- Silver stains

  Dieterle—*Legionella*

  Gomori methenamine—*Pneumocystis*, fungi

- Acid fast (Ziehl-Neelsen or Kinyoun)

  Mycobacterium, *Nocardia* (partially AF), *Cryptosporidium*, *Isospora*, *Cyclospora*, and *Microsporidia* (oocysts in feces)

- India ink—*Cryptococcus* (if negative not a reliable diagnostic method)
- Calcofluor white—fungi
- Giemsa

  Blood protozoa (*Plasmodium, Babesia, Trypanosoma, Leishmania)*

  Histoplasma capsulatum in RES cells

Name Tests

Tests	Disease
PPD or Tuberculin (Mantoux)	TB
Lepromin	Leprosy
Fungal skin tests	Clinically valuable only to demonstrate exposure or anergy
CAMP test	*Strept agalactiae* carriers
Elek test	Toxin producing *C. diphtheriae* strains
Weil-Felix	Rickettsia (with *Proteus* strain OX antigens)

Unusual Growth Requirements

Haemophilus (most species require one or both)

- **X factor** = protoporphyrin IX, the precursor of **hemin**
- **V factor** = NAD (nicotinamide dinucleotide) or NADP

Mycoplasma

- **Cholesterol**

Salt (halophilic organisms)

- ***Staph aureus*** will grow on high salt media.
- Group D enterococci will grow on 6.5% NaCl.
- ***Vibrio*** species requires NaCl to grow and grows at 6.5%.

Cysteine requirement for growth

- Four Sisters Ella of the Cysteine Chapel (mnemonic by M. Free) *Francisella, Legionella, Brucella,* and *Pasteurella*

Cultures that must be observed for a long time

- *Mycobacterium tuberculosis* and all non-tuberculous mycobacteria except rapid growers
- *Mycoplasma pneumoniae*
- Systemic fungal pathogens (*Blastomyces, Histoplasma,* and *Coccidioides* in U.S.)

TREATMENT/PREVENTION

Treat Prophylactically

- *Neisseria meningitidis* (household and day care contacts—vaccination also used in outbreaks)
- *Mycobacterium tuberculosis* with a recent skin test conversion or known household (i.e., significant) exposure; or persons under 35 with a positive skin test who have never been treated
- *Haemophilus influenzae* B (unvaccinated household contacts <6 years old)—also vaccinate
- *Neisseria gonorrhoeae* (sexual contacts)
- *Treponema pallidum* (sexual contacts)

- *Yersinia pestis*
- Neonatal eyes (*Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum*)

Vaccines Available in the U.S.

Inactivated vaccines (RIP-A; Rest In Peace Always)

- Rabies
- Influenza virus
- Salk polio (killed)—all primary vaccinations in U.S., including IC patients
- Hepatitis A
- Japanese encephalitis and several other encephalitis vaccines
- *Vibrio cholerae*

Live, attenuated vaccines

- *Francisella tularensis*
- Measles (rubeola)
- Rubella
- Mumps (killed vaccine available for IC patients)
- Sabin polio (oral)
- Smallpox
- Yellow fever
- Varicella-Zoster
- Rotavirus

Live, Pathogenic Virus (in enteric-coated capsules)

- Adenovirus

Toxoid: Chemically Modified Toxin—Vaccines

- Tetanus
- Diphtheria
- Pertussis toxoid (in DTaP)

Subunit Vaccines

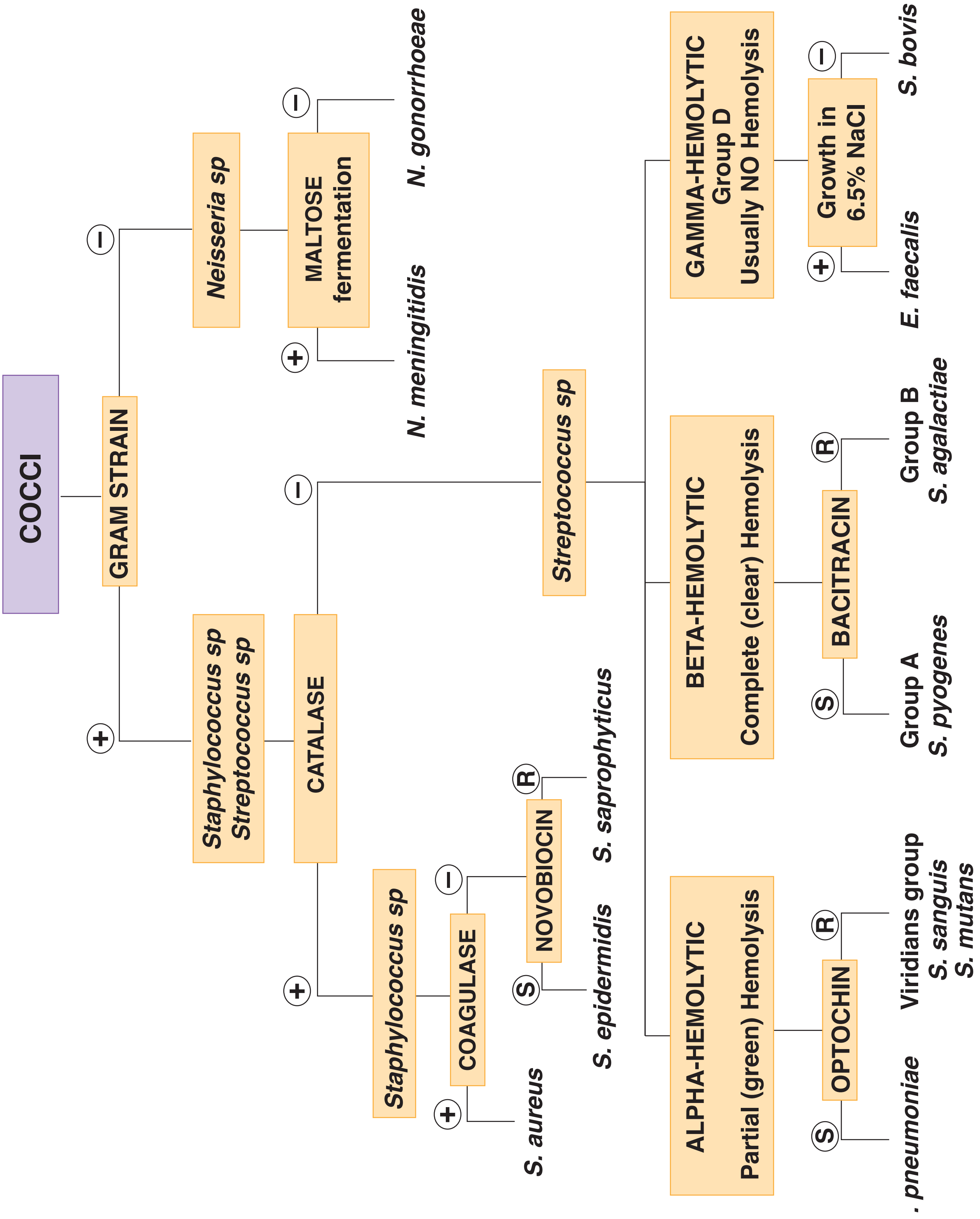
- *Haemophilus*—purified capsular polysaccharide conjugated to protein
- *Neisseria meningitidis*—capsular polysaccharides, pediatric version is conjugated to protein
- Pneumococcal—capsular polysaccharide (7 and 23 serotypes) (pediatric version is conjugated to protein)

Recombinant Vaccines

- Hepatitis B—HBsAg (produced in yeast)
- Human papilloma virus vaccine, 4 capsid proteins

Reference Charts and Tables

APPENDIX I


COCCI
GRAM STRAIN
Neisseria sp
MALTOSE fermentation
N. meningitidis
N. gonorrhoeae
Staphylococcus sp
Streptococcus sp
CATALASE
Staphylococcus sp
COAGULASE
S. aureus
NOVOBIOCIN
S. epidermidis
S. saprophyticus
Streptococcus sp
ALPHA-HEMOLYTIC
Partial (green) Hemolysis
OPTOCHIN
. pneumoniae
Viridians group
S. sanguis
S. mutans
BETA-HEMOLYTIC
Complete (clear) Hemolysis
BACITRACIN
Group A
S. pyogenes
Group B
S. agalactiae
GAMMA-HEMOLYTIC
Group D
Usually NO Hemolysis
Growth in 6.5% NaCl
E. faecalis
S. bovis

COCCI

Gram (+)

Staphylococcus sp

S. aureus
- Coagulase (+)
- Protein A
- β-Lactamase (many)
- Beta-hemolytic
- Heat-stable enterotoxins
- CAMP test hemolysin
- Salt-tolerant
- Mannitol salt agar
- Yellow/gold pigment
- Normal in nose, skin
- Toxic Shock synd
- Scalded Skin synd
- Abscesses
- Food poisoning (2–6 h)
- Acute endocarditis
- IV drug users
- Acute osteomyelitis
- DOC: MSSA: Nafcillin
- MRSA: Vancomycin
- VRSA: quinupristin

S. epidermidis
- Coagulase(-)
- Non-hemolytic
- "Plastic" adherence
- Normal in skin, nose, mouth, gut
- Subacute endocarditis in IV drug users
- Catheter, prosthetic device infections
- Novobiocin sensitive

S. saprophyticus
- Coagulase(-)
- Non-hemolytic
- UTI in sexually active adolescents
- Novobiocin resistant

Gram +

Streptococcus sp

S. pneumoniae
- Lancet-shaped
- Capsule
- Quellung (+)
- IgA protease
- Lysed by bile
- Normal in URT
- #1 for pneumonia >60 yr
- #1 for adult meningitis
- #1 Otitis media in kids
- DOC: Macrolides, ceftriaxone, amoxicillin
- ~20% Pen resist.
- Vaccine for elderly (23 serotypes)
- Vaccine pediatric (7 serotypes conjugated to protein)

Viridans group *S. sanguis* *S. mutans*
- Dextrans: Adherence dental caries
- Normal in oropharynx
- Subacute endocarditis (Post-dental work or poor dental hygiene)
- DOC: penicillin + aminoglycosides

Group A *S. pyogenes*
- Capsule of hyaluronic acid
- M protein
- Streptolysin O
- Streptolysin S
- Streptokinase
- Hyaluronidase
- Erythrogenic toxin (SPE-A)
- Rheumatic fever
- Post-Strep acute GN
- Cellulitis
- Impetigo
- DOC: Penicillin G/V

Group B *S. agalactiae*
- CAMP Test (+) (incomplete hemolysin)
- C carbohydrate
- Hydrolyzes hippurate
- Salt-tolerant
- Colonizes vagina in 15–20%
- Screen preg treat with ab. ↓ meningitis
- #1 for neonatal meningitis (↓ing)
- DOC: Ampicillin + aminoglycoside

Group D *E. faecalis*
- Enterococcus
- C carbohydrate
- Hydrolyzes bile esculin with black complex
- Salt-tolerant
- Normal in colon, gut
- UTI
- Biliary tract infections
- Post-op endocarditis
- Opportunistic infxns
- DOC: Sensitivity testing

Group D *S. bovis*
- Non-enterococcal
- C carbohydrate
- Hydrolyzes bile esculin with black complex

Gram (−)

Neisseria sp
- Kidney-bean-shaped
- Catalase (+)
- Oxidase (+), black
- IgA protease
- Endotoxin present
- Utilize glucose aerobically
- Chocolate agar
- Candle jar, CO^2

N. meningitidis
- Polysaccharide capsule
- β-Lactamase (rare)
- Pili
- Colonizes URT
- Waterhouse-Friderichsen synd.
- Tetravalent vaccine
- Ferments maltose
- DOC: cefotaxime, ceftriaxone

N. gonorrhoeae
- No capsule
- β-Lactamase
- Pili
- Probe
- Thayer-Martin medium
- DOC: Ceftriaxone PLUS Tetracycline for Chlamydia
- No vaccine
- Does not ferment maltose

Also
- *Moraxella*
- *Veillonella*
- *Eikenella*
- *Kingella*

GRAM (+) RODS

NON-SPORE-FORMING

Aerobic

Motile

L. monocytogenes

- Tumbling motility
- "Jets" from cell to cell
- Fac intracellular organism
- Beta-hemolytic
- Cold enrichment
- Sepsis
- Crosses placenta
- Meningitis
  - Renal transplant
  - Neonatal
  - Cancer
- DOC: Ampicillin and Gentamicin for IC

Non-Motile

C. diphtheriae

- Club-shaped
- "Chinese Characters"
- Exotoxin (ADP-R of eEF-2) Heart, Nerves, epithelium
- Volutin granules on Loeffler's medium
- Tellurite: black colonies
- ELEK test
- Gray pseudomembrane
- Myocarditis
- Recrnt larngl nerve palsy
- DOC: Antitoxin PLUS Erythromycin
- Toxoid vaccine

N. asteroides

- Filaments to rods
- Urease (+)
- Partially acid-fast
- Cavitary bronchopulmonary
- Multiple brain abscesses
- Mycetoma (granules)
- DOC: Sulfonamides

Anaerobic

A. israelii

- Rods with branching
- Non-motile
- Not restricted by anatomical boundaries
- Sulfur "granules" in exudates from sinus tract
- Normal in mouth and female genitourinary tract
- Cervicofacial (lumpy jaw)
- IUD-associated infections
- Solitary brain abscess
- Mycetoma
- Rx: Penicillin plus drainage

SPORE-FORMING

Aerobic

Motile

B. cereus

- Heat-stable exotoxin: vomiting increase cAMP
- Heat-labile toxin: diarrhea
- Fried rice
- Food poisoning (2–18 h)
- Symptomatic Rx

Non-Motile

B. anthracis

- Poly-D-glutamate capsule
- Spores
- In R-E cells
- Toxin:
  - Protective Ag
  - Lethal factor
  - Edema factor (an adenylate cyclase)
- Painless skin ulcer 95%
- Black eschar
- Striking local edema
- Woolsorter's disease Pneumonia
- DOC: Ciprofloxacin or doxycycline

Anaerobic

Motile

C. tetani

- Terminal spores
- Exotoxin
  - Tetanospasmin
  - Inhibits GABA
  - Glycine
- Tetanus
  - Spastic paralysis
- DOC: hyperimmune globulin, Penicillin PLUS spasmolytic
- Toxoid vaccine

C. botulinum

- Neurotoxin
  - heat-labile
  - Blocks ACh release
- Flaccid paralysis
- Canned-food poisoning
- Trivalent antitoxin
- DOC: antitoxin PLUS Penicillin
- Infant botulism human hyperimmune serum, no drugs

C. difficile

- Nosocomial
- Diarrhea to pseudomembranous colitis
- Antibiotic (Clindamycin) usage
- Yellow plaques
- Colon
- Toxins A + B
- DOC: Change or stop antibiotic

Non-Motile

C. perfringens

- Subterminal spore
- Alpha lecithinase= Phospholipase C
- Enterotoxin
- Stormy fermentation
- Double-zone hemolysis
- Egg yolk agar
- Normal in colon
- Food poisoning
- Gas gangrene
- Myonecrosis
- High mortality
- DOC: Penicillin G ± Clindamycin PLUS debridement

GRAM (-) RODS & SPIROCHETES

Facultative Anaerobes

AEROBES

B. pertussis
- Adhesion to cell via hemagglutinin and pertussis toxin
- Adenylate cyclase txn (local edema)
- Tracheal toxin
- Dermanecrosis toxin
- Endotoxin - Lipid X, A ADP-R of GNBP
- Bordet-Gengou agar
- Regan-Lowe agar
- Whooping cough
- DOC: Erythromycin
- Vaccine toxoid and filamentous hemagglutinin

Brucella sp
- In R-E cells
- Endotoxin
- Requires CYS, CO_2
- Unpasteurized milk
- Undulant Fever
  - Bang's disease
  - Malta fever
- *B. abortus*
  - cattle, mild
- *B. suis*
  - pigs
  - suppurative, chronic
- *B. melitensis*
  - goats
  - severe, acute
- DOC: rifampin and doxycycline

F. tularensis
- In R-E cells
- Requires CYS
- *Dermacentor* tick bite
  - Transovarian trans.
- Aerosol
- Rabbits, rodents
- Granulomatous rxn
- Tularemia - AR, MO, TX
- Live, attntd vaccine
- DOC: Streptomycin

L. pneumophila
- Water-loving air conditioning
- Requires CYS & Fe
- Buffered Charcoal Yeast agar
- Dieterle silver stain
- Stains poorly Gram (-)
- Atypical pneumonia
- Mental confusion
- Diarrhea
- DOC: Erythromycin
- Not contagious

P. aeruginosa
- Slime-layer
- Grape-like odor
- Exotoxin A:
  - ADP-R of eEF-2
  - Liver
- Oxidase (+)
- Pigments
  - pyocyanin, pyoverdin
- Transient colonization
  - In 10% of normal pop
- Osteomyelitis in drug abusers
- Pneumonia in cystic fibrosis
- Nosocomial infections
  - Burn patients
  - Neutropenic patients
- Ecthyma gangrenosum
- DOC: Penicillin PLUS Aminoglycoside

ANAEROBES

Bacteroides sp
- *B. fragilis* - obligate
- Modified LPS, capsules
- Predominant colonic flora
- Normal in oropharynx, vagina
- Predisposing factors:
  - surgery, trauma
  - chronic disease (cancer)
- Septicemia, peritonitis
  - aspiration pneumonia
- *Prevotella melaninogenica*
  - Human oropharynx
- Fusobacterium (combined w/ *Treponema microdentium)*
  - Vincent's angina
  - Trench mouth
- DOC: Metronidazole OR Clindamycin OR Cefoxitin

SPIROCHETES
- Thin-Walled
- Spiral-Shaped
- Axial Filaments
- Jarisch-Herxheimer Rxn

Treponema sp
- *T. pallidum* - Syphilis
  - Obligate parasite
- 1°- PAINLESS chancre, infectious
- 2°- Rash infectious
- 3°- Gummas, CVS, CNS
- Congenital: stillbirths, malformed
- VDRL & RPR - Screening tests
- Reagin ab - xrxn with Cardiolipin
- FTA-ABS (immunofluorescence) specific test
- Dark-field microscopy
- DOC: Benzathine Penicillin

Borrelia sp
- Microaerophilic
- Giemsa stain
- *B. burgdorferi*
  - Lyme disease
    - (*I. scapularis*), *I. pacificus*
  - Reservoirs: mice, deer
  - CT, WI, CA
  - Erythema Migrans
    - Target lesions
- *B. recurrentis*
  - Relapsing fever
  - Vector: body louse
  - Antigenic variation
- DOC: Penicillin or azithromycin

Leptospira sp
- Dark-field microscopy
- Contaminated water
  - Animal urine
- Fever, jaundice, uremia
- Non-icteric Leptospirosis
  - Meningitis - No PMN in CSF
  - uveitis, rash
- Icteric Leptospirosis
  - Weil's disease
  - Renal failure, myocarditis
- DOC: Penicillin G or doxycycline

Other Gram (–) RODS

FACULTATIVE ANAEROBES

P. multocida
- Requires CYS
- Animal bites (cats & dogs)
- Cellulitis with Lymphadenitis
- DOC: Amoxicillin/Clav (prophylaxis)

H. influenzae
- Polyribitol capsule
- Quellung (+)
- IgA protease
- Requires X (Hemin), V (NAD)
- Normal in nasopharynx and conjunctiva
- Pathogenic in kids: type B
- Meningitis in 1–2 yr
- Otitis media, pneumonia
- Acute epiglottitis
- DOC: Cefotaxime/Ceftriaxone
- Prev: HIB vaccine, Rifampin

Vibrio sp
- Polar flagella, comma-shaped
- Enterotoxin (Choleragen) ADP-R, increase cAMP
- Catalase (+), Oxidase (+)
- Alkaline culture (TCBS)
- Classic cholera O_1
- Biotypes: El Tor, Cholerae
- Rice-water stools
- Most severe dehydration
- Rx: fluid & electrolytes, tetracycline for contacts
- *V. parahaemolyticus*
  Catalase (-), salt-tolerant
  Raw seafood
- *V. vulnificus*
  Brackish water • Oysters
  Cellulitis, Septicemia
  DOC: tetracycline

Campylobacter sp / *Helicobacter* sp
- Microaerophilic
- Polar flagella
- Comma or S-shaped
- Oxidase (+), Catalase (+)
- Skirrow's agar CO_2
- Invasive
- ***H. pylori***
  37˚C, urease(+)
  gastritis, ulcers
  carcinoma
- DOC: omeprazole + amoxicillin + clarithromycin
- ***C. jejuni***
  42˚C enterocolitis
  #1 bacterial diarrhea U.S.A.
- "Gull-Wings"
- *C. fetus* escapes GIT
- DOC: Erythromycin, fluoroquinolones

Also:
- *Gardnerella*
- *Capnocytophaga*
- *Actinobacillus*
- *Cardiobacterium*

ENTEROBACTERIACEAE
- Ferment Glucose
- Oxidase (-), Catalase (+)
- Reduce Nitrates to Nitrites

Lactose-Fermenting

E. coli
- Normal in colon
- #1 for UTI
  P-pili, X-Adhesins
- Nosocomial infections
- Neonatal meningitis (K_1)
- ETEC • Traveler's Drha
  Toxins: LT, ST
- EIEC • Invasive
- EHEC • VTEC 0157:H7
  Hemorrhagic colitis
  Hemolytic uremic S
  Does not ferment Sorbitol
- EPEC
  Plasmid-coded EAF
- EAEC
  Fimbriae/biofilm
- DAEC
  Infants
  Bacteria in microvilli
- DOC: Ampicillin or
  Sulfonamides
  Cephalosporins

K. pneumoniae
- Capsule
- Quellung (+)
- Pneumonia
  Currant jelly septum
  Chronic lung disease
  Alcoholism
  Aspiration
- UTI
  Nosocomial
  Catheterization
- DOC: Cephalosporin +/– aminoglycoside

Non-Lactose-Fermenting

Motile and H_2S-Producing

Proteus sp.
- Swarming motility
- Indole (+), Urease (+)
- UTI, Septicemia
- Staghorn calculi
- DOC: Fluoroquinolones

Salmonella enterica subsp.
- Antigens: Vi, O, H
- EMB/MacConkey
- Predisposing factors
  High gastric pH
  Gastrectomy
- Widal test (O, H ag)
- Osteomyelitis in Sickle Cell disease
- ***S. enterica* subsp. *typhi***
  No animal res.
  No H_2S produced
  Invasive (R-E) cells
  Rose spots
- DOC: fluoroquinolones or cephalosporins
- ***S. enterica* subsp. *enteritidis***
  Poultry, reptiles
  Rx: Symptomatic
- DOC for invasive: ampicillin, cephalosporins

Non-Motile and Non-H_2S-Producing

Shigella sp
- No H Antigens
- Invasive
- Shigatoxin
  Nicks 60S SU
  Neurotoxin
  Cytotoxin
  Enterotoxin
- Enterocolitis
- Bloody diarrhea
- DOC: fluid and electrolytes
  FQ, Azithro

Y. pestis
- Coagulase (+)
- V&W antigens
- Safety-pin appearance (bipolar staining Wayson's stain)
- Wild rodents
- Flea bite
- Southwest U.S. (Sylvatic)
- Bubonic plague
  fever, buboes,
  conjunctivitis
- Pneumonic plague
- DOC: Aminoglycosides PLUS Quarantine (72 h)
- ***Y. enterocolitica***
  Cold growth
- Heat-stable toxins

Poorly Gram-Staining Organisms*

ACID FAST — Mycobacteria

M. tuberculosis
- Gram (+) wall but doesn't stain due to waxy CW
- Acid fast, obligate aerobe
- Respiratory transmission
- Pathogen, contagious
- Cord factor-trehalose mycolate-inhib. WBC migration mitoch. resp./ oxid. phosphor
- Sulfatides-inhib. phagosome-lysosome fusion
- Niacin (+), catalase (+) at 37°C, (–) at 68°C
- Slow growing
- Drug resistance
- Lowenstein-Jensen medium
- DOC: isoniazid + rifampin + pyrazinamide (2 mo) then isoniazid + rifampin (4 mo)

M. avium-intracellulare
- Gram (+) wall but doesn't stain due to waxy CW
- Acid fast
- Obligate aerobe
- Soil organism
- Opportunist, non-contagious
- Pulmonary → diss infections CA pts, late AIDS pts

M. leprae
- Obligate intracellular bacterium
- Tuberculoid (CMI damage)
- Lepromatous leprosy (poor CMI)
- DOC: dapsone + rifampin + clofazimine

M. marinum
- Cutaneous lesions (fish tank granuloma)
- DOC: isoniazid, rifampin, ethambutol

SOME ATP — Rickettsias

R. rickettsii
- Obligate intracellular bacteria
- Gram-negative envelope but stain poorly
- Rocky MT Spt'd Fever-rash on wrists/ankles → trunk, palms, soles
- Vector: *Dermacentor* tick
- Reservoirs: ticks, wild rodents
- Dx: serol: 4x incr indir Fl. Ab + Weil-Felix DOC: Doxycycline

R. prowazekii
- Obligate intracellular bacteria
- Epidemic typhus
- Vector: *Pediculus* louse
- Reservoir: humans, squirrel fleas, flying squirrels

Bartonella henselae
- Cat scratch fever
- Bacillary angiomatosis in AIDS

Ehrlichia
- Ehrlichiosis
- Morulae in WBC
- DOC: doxycycline
- *E. chaffeensis*-monocytes + macrophages
- *E. phagocytophila* - PMNs
- *Ixodes* tick

NO ATP, mod. peptidoglycan — Chlamydiaceae

Chlamydia trachomatis
- Obligate intracellular bacteria
- Gram-negative envelope but stain poorly; lack muramic acid
- Elementary body-transmitted
- Reticulate body-intracellular
- Dx: serology or tissue culture growth confirmed by inclusion bodies (Fl Ab, Giemsa, iodine)

Serotypes D-K
- U.S.-Most common bacterial STD (HPV and HSV2 more common)
- Neonatal/adult inclus. conjunct, neonatal. pneumo; urethritis cervicitis, PID, infertility

Serotypes L1, 2, 3
- Lymphogranuloma venereum
- STD in Africa, Asia, S. America

Serotypes A, B, Ba, C
- Trachoma-follic conjunctivitis → conj. scarring, entropion → corneal scarring
- Leading infectious cause blindness
- DOC: Doxycycline or azithromycin

Chlamydophila pneumoniae
- TWAR agent
- Respiratory infections
- Probably very common
- Potential association with atherosclerosis
- DOC: macrolides and tetracycline

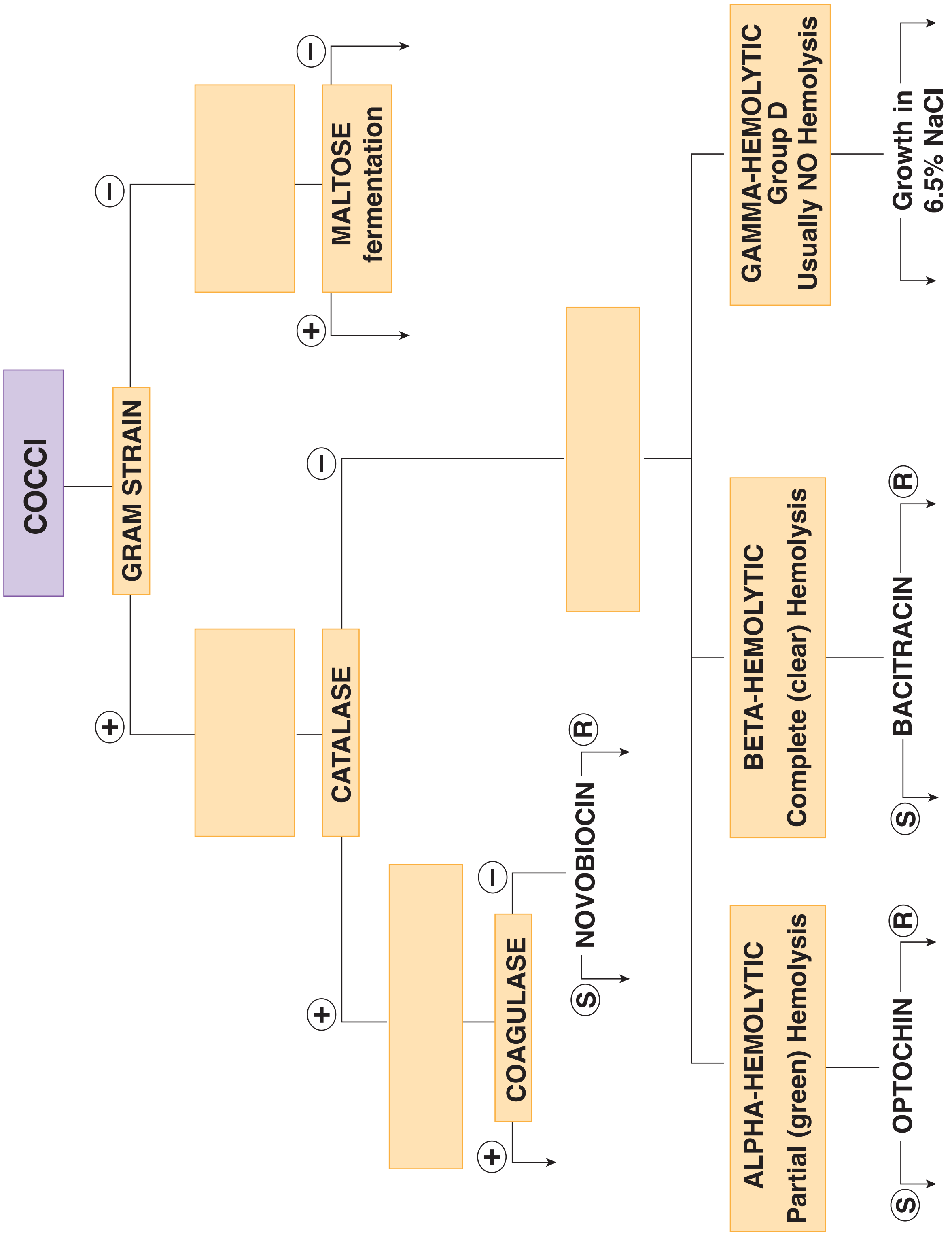
Chlamydophila psittaci
- Atypical pneumonia
- Birds (parrots)
- DOC: tetracycline

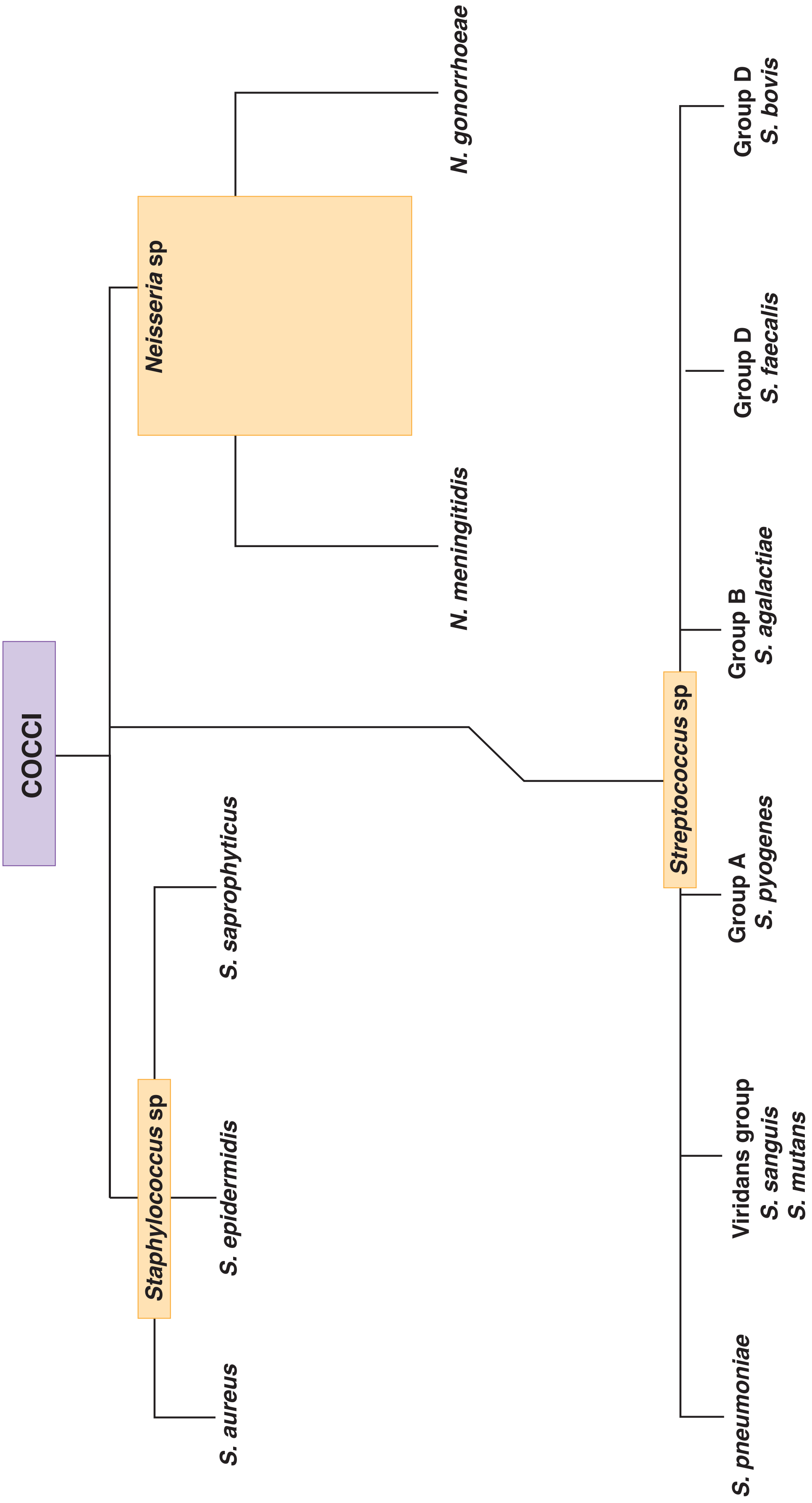
NO CELL WALL — Mycoplasmas

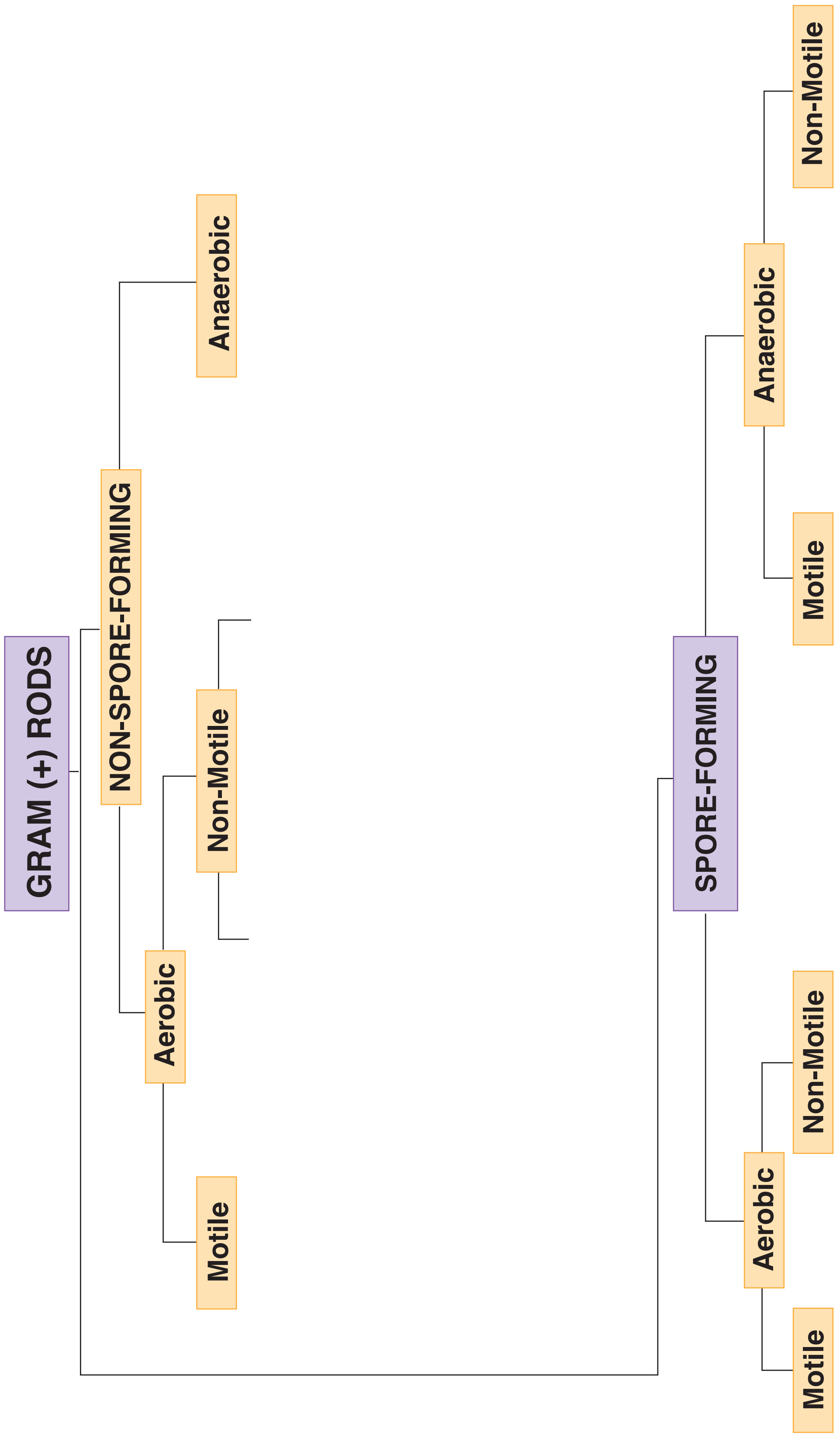
M. pneumoniae
- Lack cell wall peptidoglycan → non-Gram-staining
- Cholesterol (req'd) in membr.
- Atypical pneumonia in youth and young adults
- Free living (culturable, extracell.)
- Slow growth, special media: Mycoplasma, Eaton's or Hayflick's media-sterols+pur/pyrimidines: mulberry colonies
- Cold agglutinins in 65% cases
- No Penicillins nor Cephalosporins
- DOC: erythromycin, azithromycin

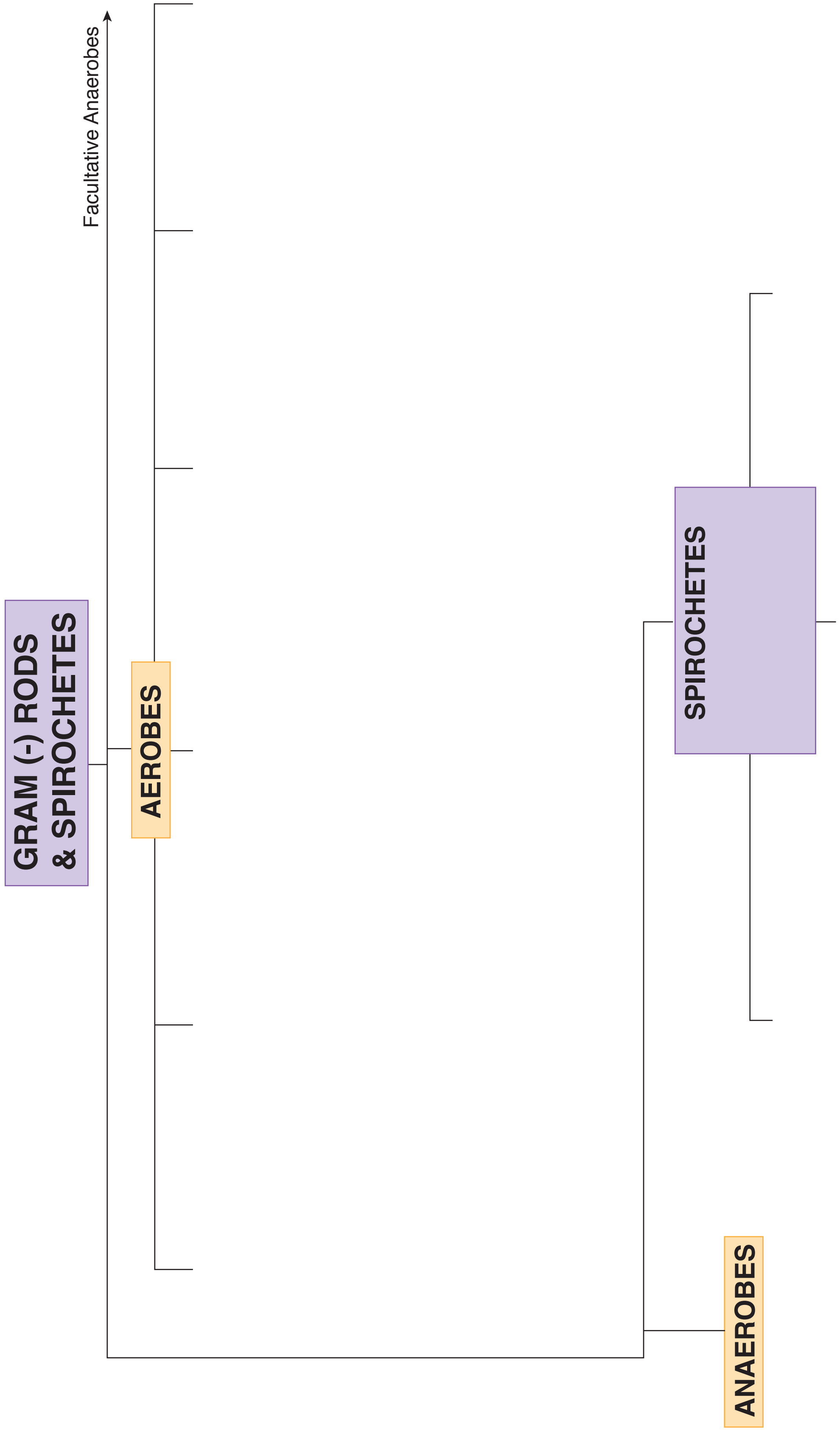
Ureaplasma urealyticum
- Urethritis, prostatitis
- Urease positive
- No cell wall
- DOC: erythromycin or tetracycline

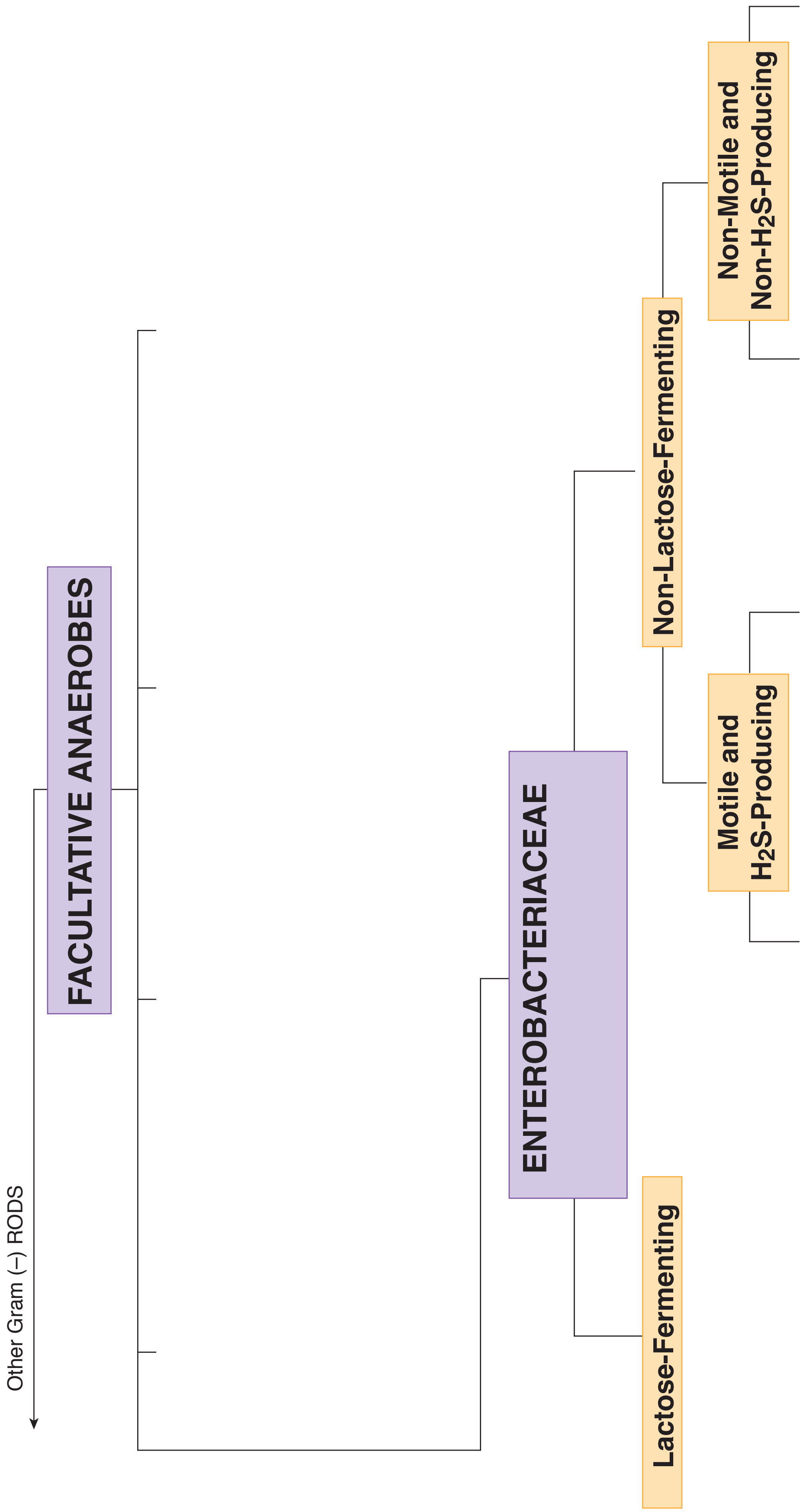
*Also note that *Legionella* and the spirochetes (*Treponema*, *Leptospira*, and *Borrelia*)—all Gram-negative—do not show up reliably with Gram stain.

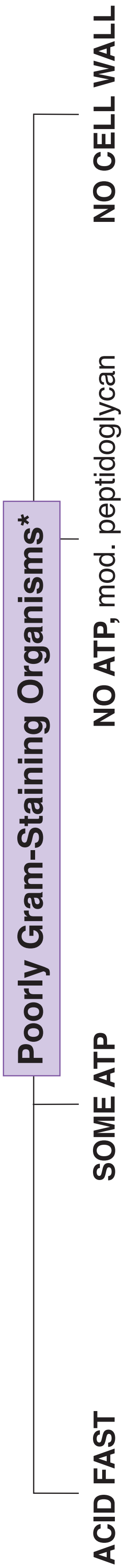

COCCI
GRAM STRAIN
MALTOSE fermentation
CATALASE
COAGULASE
NOVOBIOCIN
S
R
GAMMA-HEMOLYTIC
Group D
Usually NO Hemolysis
Growth in 6.5% NaCl
BETA-HEMOLYTIC
Complete (clear) Hemolysis
BACITRACIN
S
R
ALPHA-HEMOLYTIC
Partial (green) Hemolysis
OPTOCHIN
S
R

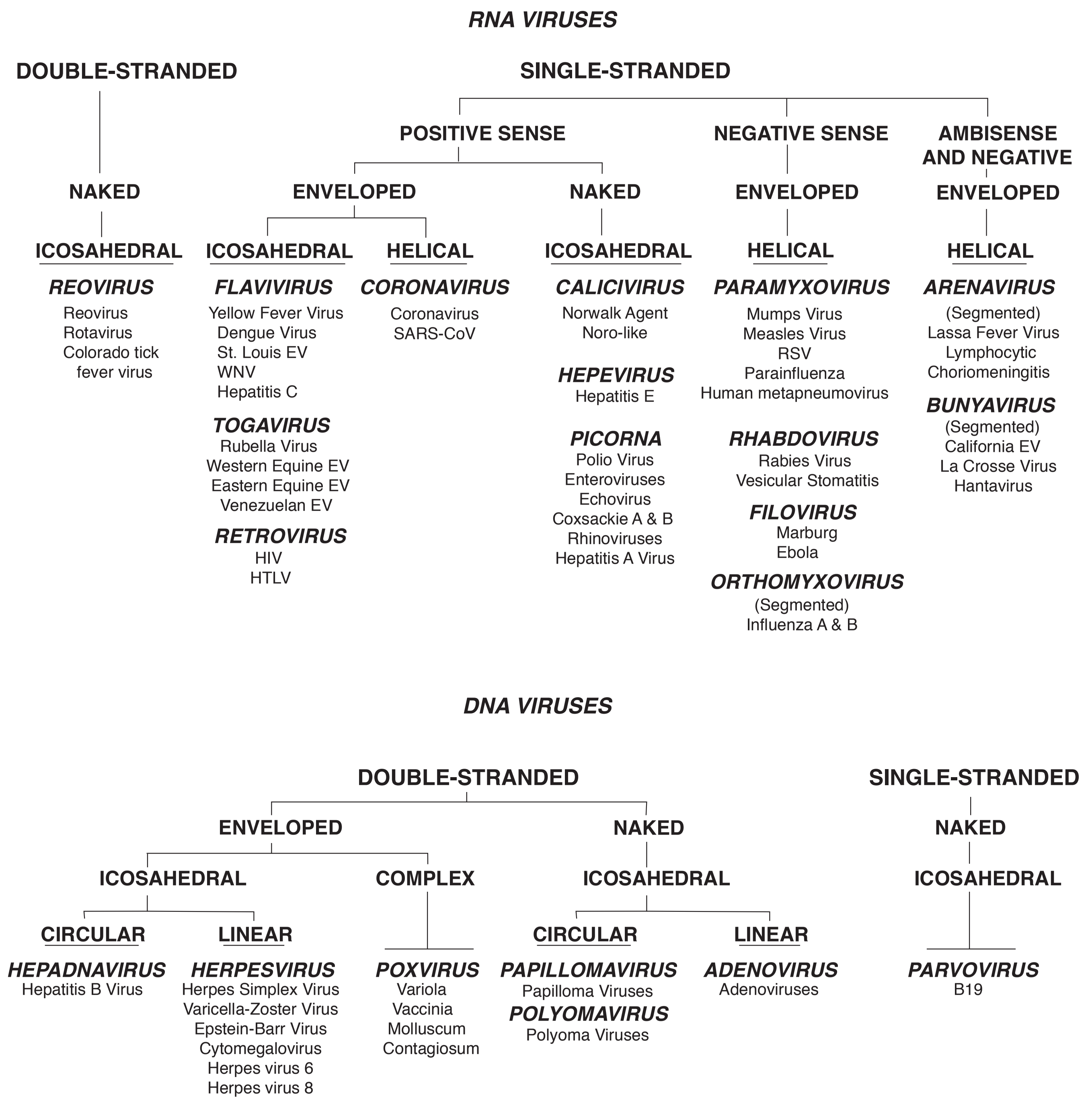

COCCI
Staphylococcus sp
S. aureus
S. epidermidis
S. saprophyticus
Neisseria sp
N. meningitidis
N. gonorrhoeae
Streptococcus sp
S. pneumoniae
Viridans group
S. sanguis
S. mutans
Group A
S. pyogenes
Group B
S. agalactiae
Group D
S. faecalis
Group D
S. bovis

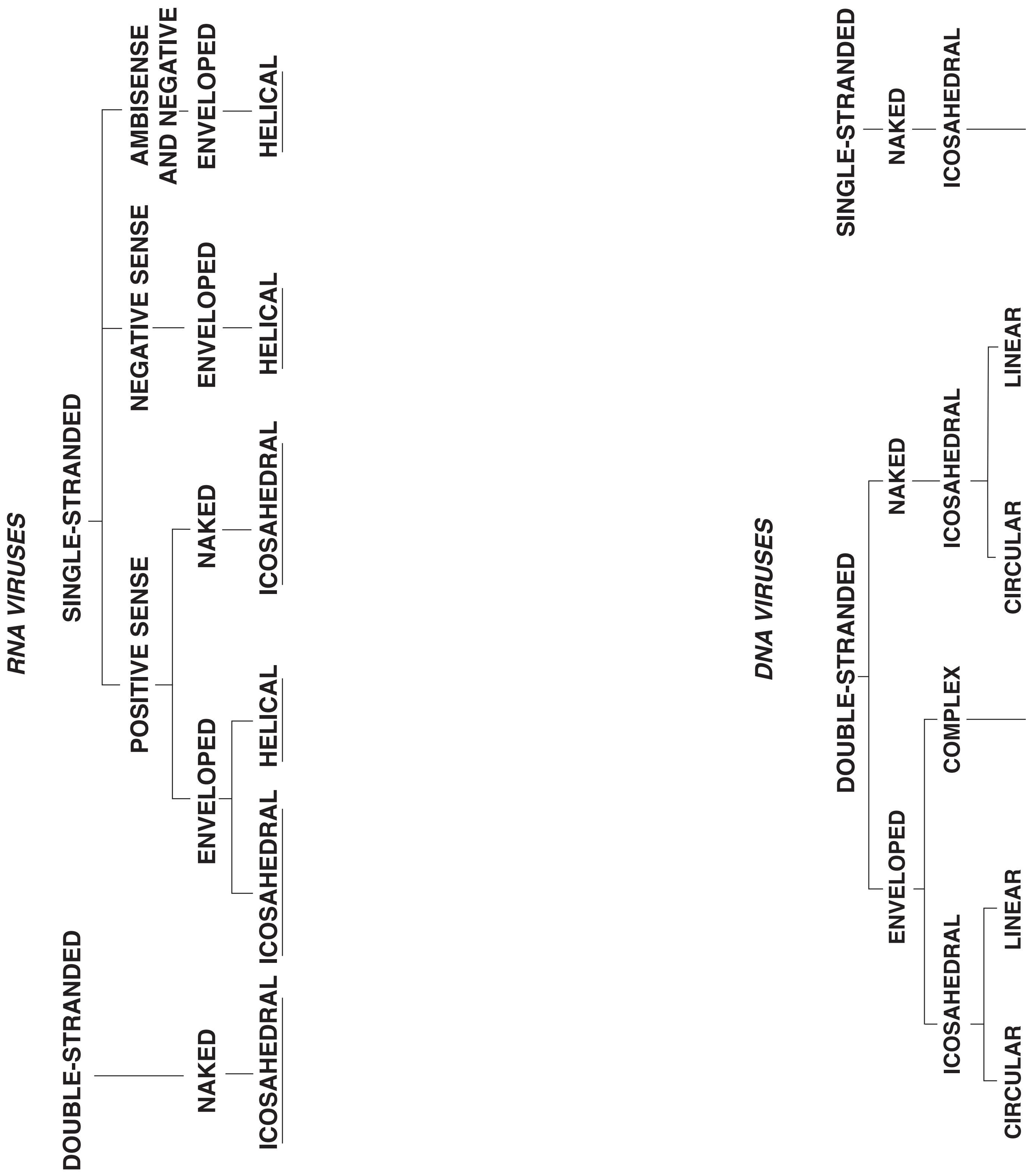

GRAM (+) RODS
NON-SPORE-FORMING
Anaerobic
Aerobic
Non-Motile
Motile
SPORE-FORMING
Anaerobic
Non-Motile
Motile
Aerobic
Non-Motile
Motile


GRAM (-) RODS & SPIROCHETES
Facultative Anaerobes
AEROBES
SPIROCHETES
ANAEROBES


FACULTATIVE ANAEROBES
Other Gram (−) RODS
ENTEROBACTERIACEAE
Lactose-Fermenting
Non-Lactose-Fermenting
Motile and
H_2S-Producing
Non-Motile and
Non-H_2S-Producing


Poorly Gram-Staining Organisms*
ACID FAST
SOME ATP
NO ATP, mod. peptidoglycan
NO CELL WALL


RNA VIRUSES
DOUBLE-STRANDED
NAKED
ICOSAHEDRAL
REOVIRUS
Reovirus
Rotavirus
Colorado tick fever virus
SINGLE-STRANDED
POSITIVE SENSE
ENVELOPED
ICOSAHEDRAL
FLAVIVIRUS
Yellow Fever Virus
Dengue Virus
St. Louis EV
WNV
Hepatitis C
TOGAVIRUS
Rubella Virus
Western Equine EV
Eastern Equine EV
Venezuelan EV
RETROVIRUS
HIV
HTLV
HELICAL
CORONAVIRUS
Coronavirus
SARS-CoV
NAKED
ICOSAHEDRAL
CALICIVIRUS
Norwalk Agent
Noro-like
HEPEVIRUS
Hepatitis E
PICORNA
Polio Virus
Enteroviruses
Echovirus
Coxsackie A & B
Rhinoviruses
Hepatitis A Virus
NEGATIVE SENSE
ENVELOPED
HELICAL
PARAMYXOVIRUS
Mumps Virus
Measles Virus
RSV
Parainfluenza
Human metapneumovirus
RHABDOVIRUS
Rabies Virus
Vesicular Stomatitis
FILOVIRUS
Marburg
Ebola
ORTHOMYXOVIRUS
(Segmented)
Influenza A & B
AMBISENSE AND NEGATIVE
ENVELOPED
HELICAL
ARENAVIRUS
(Segmented)
Lassa Fever Virus
Lymphocytic Choriomeningitis
BUNYAVIRUS
(Segmented)
California EV
La Crosse Virus
Hantavirus
DNA VIRUSES
DOUBLE-STRANDED
ENVELOPED
ICOSAHEDRAL
CIRCULAR
HEPADNAVIRUS
Hepatitis B Virus
LINEAR
HERPESVIRUS
Herpes Simplex Virus
Varicella-Zoster Virus
Epstein-Barr Virus
Cytomegalovirus
Herpes virus 6
Herpes virus 8
COMPLEX
POXVIRUS
Variola
Vaccinia
Molluscum Contagiosum
NAKED
ICOSAHEDRAL
CIRCULAR
PAPILLOMAVIRUS
Papilloma Viruses
POLYOMAVIRUS
Polyoma Viruses
LINEAR
ADENOVIRUS
Adenoviruses
SINGLE-STRANDED
NAKED
ICOSAHEDRAL
PARVOVIRUS
B19


RNA VIRUSES
DOUBLE-STRANDED
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ICOSAHEDRAL
NEGATIVE SENSE
ENVELOPED
HELICAL
AMBISENSE AND NEGATIVE
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HELICAL
DNA VIRUSES
DOUBLE-STRANDED
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ICOSAHEDRAL
CIRCULAR
LINEAR
COMPLEX
NAKED
ICOSAHEDRAL
CIRCULAR
LINEAR
SINGLE-STRANDED
NAKED
ICOSAHEDRAL

Bacterial Envelope (All the Concentric Surface Layers of the Bacterial Cell)

Envelope Structure	Gram + or –	Chemical Composition	Function
Capsule (Non-essential) = Slime layer = Glycocalyx	Both	Polysaccharide gel*	Pathogenicity factor protecting against phagocytosis until opsonized; immunogenic**
Outer membrane	Gram – only	Phospholipid/proteins: Lipopolysaccharide Lipid A Polysaccharide	Hydrophobic membrane: LPS = endotoxin Lipid A = toxic moiety PS = immunogenic portion
		Outer membrane proteins	Attachment, virulence, etc.
		Protein porins	Passive transport
Cell wall **= peptidoglycan**	Gram + (thick) Gram – (thin)	Peptidoglycan-open 3-D net of: N-acetyl-glucosamine N-acetyl-muramic acid amino acids (DAP)	Rigid support, cell shape, and protection from osmotic damage Synthesis inhibited by penicillins and cephalosporins Confers Gram reaction
	Gram + only	Teichoic acids***	Immunogenic, induces TNF-alpha, IL-1 Attachment
	Acid-fast only	Mycolic acids	Acid-fastness Resistance to drying and chemicals
Periplasmic space	Gram – only	"Storage space" between the inner and outer membranes	Enzymes to break down large molecules, (β-lactamases) Aids regulation of osmolarity
Cytoplasmic membrane = inner membrane = cell membrane = plasma membrane	Gram + Gram –	Phospholipid bilayer with many embedded proteins	Hydrophobic cell "sack" Selective permeability and active transport Carrier for enzymes for: Oxidative metabolism Phosphorylation Phospholipid synthesis DNA replication Peptidoglycan cross linkage Penicillin binding proteins (PBPs)

Definition of abbreviation: DAP, diaminopimelic acid.

* Except *Bacillus anthracis*, which is a polypeptide of poly D-glutamate.

** Except *S. pyogenes* (hyaluronic acid) and type B *N. meningitidis* (sialic acid), which are nonimmunogenic.

*** Teichoic acid: polymers of ribitol or glycerol, bound to cell membrane or peptidoglycan.

Outer Surface Structures of the Bacterial Cell

Pilus or fimbria 1. Common 2. Sex 3. Virulence	Primarily Gram –*	Glycoprotein (pilin)	Adherence to cell surfaces, including attachment to other bacteria during conjugation
Flagellum	+ and –	Protein (flagellin)	Motility
Axial filaments (internal flagellum)	Spirochetes gram –	Protein	Motility

*M-protein of group A strep described as diffuse fimbriate layer or fimbriae.

Internal Bacterial Structures*

Structure	Cell Type	Chemical Composition	Function
Nucleoid region No membrane No histones No introns	Gram + and gram –	DNA RNA Proteins	Genetic material (all essential genes) Primers, mRNA Linker proteins, polymerases
Plasmids	Gram + and gram –	DNA	Non-essential genetic material Roles in conjugation, drug resistance, toxin production
Ribosomes	Gram + and gram –	70S (protein/RNA) 30S (16S RNA) 50S (23 and 5S)	Protein synthesis
Granules (various types)	Gram + and gram –	Glycogen, lipids, polyphosphate, etc.	Storage: polymerization of molecules present in high numbers in cells reduces osmotic pressure. Volutin granules of *Corynebacterium diphtheriae* are used in clinical identification.
Endospores	Gram + *only*	Keratin coat, calcium dipicolinate	Resistance to heat, chemicals, and dehydration

*Note that there are no mitochondria or membrane-bound structures, such as chloroplasts.

Microbiology Practice Questions

GENERAL MICROBIOLOGY

1. A 21-year-old student was seen by his family physician with complaints of pharyngitis. Examination of the pharynx revealed patchy erythema and exudates on the tonsillar pillars. Throat smear showed gram-positive cocci in chains and gram-negative diplococci. He admitted to having been sexually active. What is the significance of the Gram stain smear in this case?

   (A) It provides a rapid means of diagnosing the infection
   (B) It indicates laboratory contamination
   (C) It is not useful as it is not possible to make a diagnosis this way
   (D) It strongly suggests gonococcal pharyngitis
   (E) It is evidence of infection with hemolytic streptococci and *Neisseriae*

2. Your laboratory isolates an entirely new and unknown pathogen from one of your patients, which has all the characteristics of an aerobic filamentous fungus except that the ribosomes are prokaryotic. Unfortunately, your patient with this pathogen is very ill. Which agent would most likely be successful in treating your patient?

   (A) Third generation of cephalosporins
   (B) Isoniazid
   (C) Metronidazole
   (D) Careful limited usage of Shiga toxin
   (E) Tetracycline

3. Mitochondria are missing in

   (A) Filamentous fungi
   (B) Protozoan parasites
   (C) Viruses
   (D) Yeasts
   (E) Cestodes

4. A culture isolate from a patient with subacute endocarditis is reported to be gram positive and possess a complex carbohydrate cell wall. What is the most likely taxonomic group of the causal agent?

   (A) Fungus
   (B) Parasite
   (C) Prion
   (D) Prokaryote
   (E) Virus

5. A patient with a non-healing skin lesion has that lesion biopsied to determine its cause. The pathology lab reports back that the lesion has the characteristics of a stellate granuloma. Which of the following is most likely to be true of the causal agent?

   (A) It has lipopolysaccharide.
   (B) It has pili.
   (C) It is an exotoxin producer.
   (D) It is a superantigen.
   (E) It is intracellular.

6. A cancer chemotherapy patient has to have her intravenous port revised after it becomes blocked and the catheter is found to contain bacterial contaminants. Which of the following attributes is most likely to be a factor in this pathogenesis?

   (A) Biofilm production
   (B) Ergosterol containing membrane
   (C) Peptidoglycan layer
   (D) Possession of IgA protease
   (E) Possession of pili

7. A 45-year-old female executive goes to a cosmetic surgeon with the complaint of frown lines on her forehead which she feels are negatively affecting her appearance. Rather than undergoing surgery, she opts to try injection of Botox. What is the mechanism of action of this toxin?

   (A) It blocks release of acetylcholine.
   (B) It inhibits glycine and GABA.
   (C) It is a lecithinase.
   (D) It is a superantigen.
   (E) It ribosylates eukaryotic elongation factor-2.
   (F) It ribosylates Gs.

MEDICALLY IMPORTANT BACTERIA

8. A 4-year-old boy develops several honey-crusted lesions behind his ears and on his face. The simplest test for the physician to determine the genus of bacteria responsible for this child's illness is the

   (A) catalase test
   (B) coagulase test
   (C) growth of the organism in 6.5% sodium chloride
   (D) hemolysis pattern on blood agar
   (E) polymerase chain reaction

9. An atherosclerotic 80-year-old man develops a pelvic abscess following a ruptured appendix. What is/are the most likely causal agent(s)?

(A) *Bacteroides* species and microaerophilic streptococci
(B) *Candida albicans*
(C) *Enterobacter aerogenes*
(D) *Haemophilus influenzae* group B
(E) *Streptococcus viridans*

10. A homeless, malnourished chronic alcoholic presents with severe headache and dyspnea. Physical examination reveals a disheveled man with poor hygiene. His temperature is 41.0 C (105.8 F), blood pressure is 110/78 mm Hg, and pulse is 96/minute and regular. Auscultation of the chest reveals absence of breath sounds over the left middle lung fields. A chest x-ray confirms left lobar pneumonia. Sputum stain reveals partially acid-fast bacilli with branching rods. Which of the following agents is the most likely cause?

(A) *Mycobacterium avium-intracellulare*
(B) *Mycobacterium kansasii*
(C) *Mycobacterium leprae*
(D) *Mycobacterium tuberculosis*
(E) *Nocardia asteroides*

11. A 70-year-old man presents to the emergency department with a fever of 39.7 C (103.5 F), a dry cough, tachypnea, and chest pain. History reveals he has been smoking since he was a teen. He mentions that several people at the assisted living community where he resides have had similar symptoms. A sputum sample isolated organisms that grew on buffered charcoal yeast extract agar and stained weakly gram-negative. Which of the following properties is consistent with the organism?

(A) Capsule
(B) No cell wall
(C) Optochin sensitive
(D) Requires iron and cysteine for growth
(E) Serpentine growth in vitro

12. A 33-year-old man presents to the emergency department with a fever of 39.1 C (102.5 F), facial palsy, headache, and malaise. A circular maculopapular rash was identified on the patients left shoulder; the patient was unaware of the rash. The patient likely acquired the infection via which of the following routes?

(A) Consumption of contaminated food
(B) Direct contact with fomite
(C) Arthropod vector
(D) Respiratory route
(E) Sexual contact

13. A 25-year-old man develops a high fever and swelling in the armpits and groin. Aspirates from the lymph nodes reveal gram-negative rods with bipolar staining. The patient is most likely

    (A) a farmer
    (B) from the southwestern U.S.
    (C) in the military
    (D) living in a dormitory
    (E) sexually promiscuous

14. A previously healthy 5-month-old infant presents with apparent upper body weakness including droopy eyes, head lag, drooling, and inability to sit unassisted. The most likely infectious form is

    (A) elementary body
    (B) endospore
    (C) exotoxin
    (D) reticulate body
    (E) vegetative cell

15. Sixteen residents in a retirement home have fever, malaise, and anorexia. These residents have taken their meals prepared by the same kitchen. Blood cultures from 11 of these residents grow *Salmonella enterica* subsp. *typhi*. The primary reservoir of this organism is

    (A) hen's egg
    (B) dogs and cats
    (C) turkeys
    (D) people
    (E) water

16. If a culture is inoculated to a density of 5×10^2 cells/mL at time 0 and has both a generation time and lag time of 10 minutes, how many cells/mL will there be at 40 minutes?

    (A) 1.5×10^3
    (B) 2×10^3
    (C) 4×10^3
    (D) 6×10^3
    (E) 4×10^6

17. A 6-year-old girl had crashed on a toboggan ride and complained of pain in the perineal area. Exam showed only bruising of the area. Two days later, she develops fever, prostration, discoloration of the buttock, and blebs of the skin in the area. After admission to the hospital, she develops progressive involvement of the leg, thigh, and buttock with extension to the lower abdomen. She goes into shock and dies before surgery could be performed. At autopsy, a 1-inch piece of wood is found in the perineum, which had perforated the anus. The most likely causal agent

 (A) requires an elevated oxidation reduction potential
 (B) is a gram-negative coccobacillus
 (C) is a marked lecithinase producer
 (D) is nonhemolytic on blood agar
 (E) is nonfermentative

18. A 71-year-old man is admitted from his extended care facility (nursing home) because of recent aggravation of an exfoliative skin condition that has plagued him for several years. He had been receiving a variety of topical antibiotic regimens over the last year or two. He now has a temperature of 38.9 C (102 F). The skin of upper chest, extremities, and neck shows erythema with diffuse epidermal peeling and many pustular lesions. Cultures obtained from these lesions were reported back from the laboratory as yielding a gram-positive organism that is highly salt (NaCl) tolerant. What lab result is used to confirm the species of the causal agent?

 (A) Bacitracin sensitivity
 (B) Bile solubility
 (C) Catalase production
 (D) Coagulase production
 (E) Optochin sensitivity

19. Eight of 10 family practice residents who had a potluck four days ago now have diarrhea with abdominal cramps, general malaise, and fever ranging from 37.5 to 38.7 C (99.5 to 101.6 F). Stools from three residents are blood tinged. Laboratory studies revealed the causal agent was a microaerophilic gram-negative, curved rod with polar flagella often in pairs to give a “seagull” appearance. It grew on special media at 42 C (107.6 F). The original contamination probably was found in

 (A) poultry
 (B) improperly canned food
 (C) fried rice
 (D) fish
 (E) vegetables

20. A 19-year-old man was brought to the emergency department by his dorm mate with a petechial rash, headache, nuchal rigidity, and vomiting. Which of the following describes the most likely causal agent?

    (A) Gram-negative coccus, capsule, ferments maltose
    (B) Gram-negative coccus, ferments glucose only
    (C) Gram-negative coccobacillus, capsular serotype b
    (D) Gram-positive coccus, alpha hemolytic, optochin sensitive
    (E) Gram-positive rods, growth at 4 C (39.2 F)

21. A 70-year-old woman is brought to the emergency department by her spouse with complaints of shortness of breath and fever. Physical examination revealed a fever of 39.4 C (103.0 F), hypotension, and a diastolic murmur. History revealed a cardiac valve replacement five years earlier. Three consecutive blood cultures taken during febrile periods revealed gram-positive cocci that were catalase-positive and coagulase-negative. Which of the following organisms is the most likely cause?

    (A) *Enterococcus faecalis*
    (B) *Kingella kingae*
    (C) *Staphylococcus aureus*
    (D) *Staphylococcus epidermidis*
    (E) *Staphylococcus saprophyticus*

22. What is the structure that is found in gram-negative but not in gram-positive bacteria?

    (A) Capsule
    (B) Cell wall
    (C) Cytoplasmic membrane
    (D) Endospore
    (E) Outer membrane

23. A tourist who recently returned from a trip to Peru goes to her physician complaining of persistent high fever, malaise, and constipation that persisted over a week. She recalls that the fever began slowly and climbed to 41.0 C (105.8 F). A physical exam reveals an enlarged spleen and tender abdomen with rose-colored spots. Laboratory isolation of a bacterium that produces H_2S and is motile is revealed. Which organism is the most likely cause of her condition?

    (A) EHEC
    (B) ETEC
    (C) *Salmonella enterica* subsp. *enteritidis*
    (D) *Salmonella enterica* subsp. *typhi*
    (E) *Shigella dysenteriae*

24. A 5-year-old child of an Eastern European immigrant family is brought to your pediatric clinic. The child is afebrile, but weak and exhausted from a week of paroxysmal coughing with inspiratory whoops, frequently associated with vomiting. The parents profess religious objections to childhood vaccinations, but permit withdrawal of a blood sample, which reveals a lymphocytosis of 44,000/mm^3. Production of lymphocytosis, insulin secretion, and histamine sensitization are all results of which attribute of this organism?

(A) Motility
(B) Adenylate cyclase toxin
(C) Beta-hemolysin
(D) Anaerobic growth
(E) Pertussis toxin
(F) Filamentous hemagglutinin

25. The clinical laboratory reports the presence of 0157:H7 strains of *E. coli* in the bloody stools of six children ages 3–5 who attended a local petting zoo. These young children would be at an increased risk for developing

(A) buboes
(B) hemolytic uremic syndrome
(C) infant botulism
(D) renal stones
(E) rice water stools

26. A 65-year-old man develops pneumonia. The organisms isolated from the sputum are gram-positive cocci that are alpha hemolytic on blood agar and sensitive to optochin. Which structure of the causal agent provides protection against phagocytosis?

(A) Capsule
(B) Catalase
(C) Coagulase
(D) M protein
(E) Teichoic acid

27. A 68-year-old woman on chemotherapy for leukemia has developed sepsis due to an infection with *Escherichia coli*. The following day the patient develops septic shock and dies. The structure on the bacterium most likely responsible for causing septic shock in this patient is

(A) capsule
(B) lipopolysaccharide
(C) pili
(D) spore
(E) teichoic acid

28. A 12-year-old boy from North Carolina presents to the emergency department with rash, fever, and severe headache that began three days ago. The rash began on his arms and legs and then spread to the trunk. The pediatrician notes conjunctival redness, and lab tests reveal proteinuria. Which of the following events likely led to the child's illness?

    (A) Cutting himself while butchering rabbits
    (B) Eating undercooked meat
    (C) Hiking in the woods
    (D) Kissing
    (E) Not washing his hands

29. A 10-year-old child develops glomerulonephritis a week after he was treated for a sore throat. The causal agent is identified by serotyping of the

    (A) capsule
    (B) M proteins
    (C) outer membrane proteins
    (D) pili
    (E) teichoic acids

30. An 8-year-old boy presents to the emergency department with vomiting and a severe cough in which he can't catch his breath. His vaccination history is incomplete. Physical exam reveals fever and conjunctival injection. A nasopharyngeal aspirate grew gram-negative coccobacilli on Bordet-Gengou media. What is the mechanism of action of the toxin involved?

    (A) ADP ribosylation of eukaryotic elongation factor 2 (eEF-2)
    (B) ADP ribosylation of G_i
    (C) ADP ribosylation of GTP-binding protein
    (D) Blocks release of acetylcholine
    (E) Blocks release of inhibitory transmitters GABA and glycine

31. What is the typical means of transmission of a toxin that blocks the release of inhibitory transmitters GABA and glycine?

    (A) Eating home-canned foods
    (B) Fecal-oral, travel to foreign country
    (C) Infant given honey during the first year of life
    (D) Puncture wound
    (E) Respiratory, with incomplete vaccination history

32. An infant presents to the emergency department due to difficulty breathing, constipation, and anorexia. Upon examination, the physician notes flaccid paralysis. A toxin screen of the stool identified the agent. What is the mechanism of action of the toxin?

    (A) ADP ribosylation of eukaryotic elongation factor 2 (eEF-2)
    (B) ADP ribosylation of G_i
    (C) ADP ribosylation of GTP-binding protein
    (D) Blocks release of acetylcholine
    (E) Blocks release of inhibitory transmitters GABA and glycine

33. A 10-year-old girl with an incomplete vaccination history presents to her pediatrician with a fever of 38.6 C (101.5 F), sore throat, malaise, and difficulty breathing. Physical examination reveals cervical lymphadenopathy and a gray, leathery exudate in the rear of the oropharynx. The area bleeds profusely when disturbed with a tongue depressor. Which of the following correctly describes the causal agent?

    (A) Gram-negative rod; toxin that inhibits protein synthesis
    (B) Gram-negative rod; toxin that increases cAMP
    (C) Gram-positive aerobic rod; toxin that inhibits protein synthesis
    (D) Gram-positive anaerobic rod; toxin that inhibits protein synthesis
    (E) Gram-positive aerobic rod; toxin that increases cAMP

34. A 38-year-old man who recently visited India on business presents to the emergency department with profuse watery diarrhea flecked with mucus, and severe dehydration. Which of the following correctly describes the causal agent?

    (A) Gram-negative curved rod; toxin that increases cAMP
    (B) Gram-negative curved rod; toxin that inhibits protein synthesis
    (C) Gram-negative rod; toxin that increases cAMP
    (D) Gram-negative rod; toxin that inhibits protein synthesis
    (E) Intoxication with a heat labile toxin that blocks the release of acetylcholine

35. A 30-year-old man presents to his physician with complaints of midepigastric pain. He describes the pain as moderate, occasionally waking him at night, and improving immediately following meals. A urease breath test was positive. Which of the following correctly describes the causal agent?

    (A) Gram-negative curved rod; microaerophilic
    (B) Gram-negative rod; aerobic
    (C) Gram-negative rod; facultative anaerobe
    (D) Gram-positive rod; aerobic
    (E) Gram-positive rod; microaerophilic

36. A 13-year-old girl presents to her pediatrician with fever, malaise, and a sore throat. Physical examination reveals a fever of 39.4 C (103.0 F), cervical lymphadenopathy, and pharyngeal erythema. A swab is taken from some of the tonsillar exudate and cultured on blood agar. Culture reveals beta hemolytic, gram-positive cocci, and a rapid antigen test is positive. What is the major component that protects the causal agent from osmotic damage?

(A) Lipopolysaccharide
(B) Peptidoglycan
(C) Phospholipids
(D) Polysaccharide
(E) Teichoic acid

37. A 27-year-old woman, after returning home from her honeymoon, has developed urinary frequency, dysuria, and urgency. Her urine is grossly bloody. Which lab data are most likely to define the causal agent?

(A) A gram-negative diplococcus, which is oxidase positive but does not ferment maltose
(B) A gram-positive coccus, which is catalase positive and coagulase negative
(C) An optochin-resistant, catalase-negative, gram-positive coccus
(D) A gram-positive bacillus grown on a low oxidation-reduction medium
(E) A gram-negative bacterium capable of reducing nitrates to nitrites

38. Two days after eating a meal that included home-canned green beans, three people developed various degrees of visual problems, including double vision and difficulties focusing. Describe the Gram reaction of the organism most likely to be isolated from the leftover beans and lab findings which would be used in its identification.

(A) A gram-positive coccus which is catalase-positive and grows in a high salt environment
(B) A gram-positive aerobic bacillus which sporulates
(C) A gram-positive coccus which is catalase-negative and optochin-resistant
(D) A gram-positive bacillus grown on a low oxidation-reduction medium
(E) A gram-negative bacillus capable of reducing nitrates to nitrites

39. A 16-year-old has pneumonia with a dry, hacking cough. The x-ray pattern shows a light, diffuse infiltrative pattern. The most likely organism producing these symptoms is

(A) A non-Gram–staining bacterium requiring sterols
(B) A bacillus showing granules when stained with methylene blue
(C) A bacitracin-sensitive, catalase-negative gram-positive coccus
(D) A coagulase positive, gram-positive, catalase positive coccus in clusters
(E) A gram-positive bacillus grown on a low oxidation-reduction medium

40. A 7-day-old infant presents to the emergency department with a fever, poor feeding, and a bulging fontanelle. During her physical examination, she begins to convulse. A Gram stain of the CSF reveals gram-positive rods. Which of the following organisms is the most likely causal agent?

(A) *Escherichia coli*
(B) *Haemophilus influenzae*
(C) *Listeria monocytogenes*
(D) *Neisseria meningitidis*
(E) *Streptococcus agalactiae*

41. A 55-year-old woman had her rheumatic heart valve replaced with a prosthetic valve. Six blood cultures became positive after three days of incubation. An optochin-resistant, catalase-negative gram-positive coccus that was alpha-hemolytic was isolated. What was the most likely causal agent?

(A) *Streptococcus viridans*
(B) *Pseudomonas aeruginosa*
(C) *Serratia marcescens*
(D) *Staphylococcus aureus*
(E) *Streptococcus pneumoniae*

42. A surgical patient develops an abdominal abscess. The abscess was drained, and culture reveals a polymicrobial infection. The predominant organism identified is a gram-negative anaerobic rod. Which of the following is the most likely causal agent?

(A) *Bacteroides fragilis*
(B) *Escherichia coli*
(C) *Pseudomonas aeruginosa*
(D) *Staphylococcus aureus*
(E) *Staphylococcus epidermidis*

43. A 40-year-old homeless man presents to the emergency department with fever and night sweats, coughing up blood. Acid-fast bacilli are identified in his sputum. Which of the following virulence factors allows the causal agent to inhibit phagosome-lysosome fusion to survive intracellularly?

(A) Cord factor
(B) Calcium dipicolinate
(C) Peptidoglycan
(D) Sulfatides
(E) Tuberculin

44. A 28-year-old woman presents to her gynecologist with complaints of a malodorous vaginal discharge. Upon examination the physician notes a thin, gray vaginal discharge with no vaginal redness. A whiff test was positive for an amine odor. Which of the following is consistent with this case?

(A) Clue cells
(B) Gram-negative diplococci in PMNs
(C) Koilocytic cells
(D) Owl-eye inclusions
(E) Tzanck smear

45. Several postal workers come down with symptoms of dyspnea, cyanosis, hemoptysis, and chest pain. Chest x-ray reveals mediastinal widening. Sputum cultures are negative for all routine respiratory pathogens. Serology correctly identifies the causal agent. Which of the following structures is possessed by the causal agent?

(A) Elementary body
(B) Endotoxin
(C) Periplasmic space
(D) Reticulate body
(E) Spore

46. A 25-year-old man gets into a fight at the local bar and punches another patron in the mouth. The following day his fist becomes infected and he visits a local urgent care center. Exudate from the wound is cultured on blood and chocolate agar and reveals gram-negative rods that have a bleach-like odor. Which of the following agents is the most likely cause?

(A) *Actinobacillus actinomycetemcomitans*
(B) *Cardiobacterium hominis*
(C) *Eikenella corrodens*
(D) *Pseudomonas aeruginosa*
(E) *Kingella kingae*

47. A 45-year-old woman presents to the emergency department with intense pain in her lower back and a burning sensation upon urination. A urine culture was taken and plated on MacConkey agar. Gram-negative rods that did not ferment lactose were identified. Which virulence factor of the causal agent is most important to pathogenesis?

(A) Capsule
(B) Catalase
(C) Coagulase
(D) Exotoxin
(E) Urease

48. A 70-year-old man is hospitalized for an infection and treated with clindamycin. The patient improves and returns to his nursing home. Two weeks later he is rushed to the emergency room with fever and loose, mucoid green stools. The diarrhea is voluminous, and he is having severe abdominal pain. Sigmoidoscopy of his colon reveals yellow-white plaques. What is the single most likely event/factor that contributed to this patient's current illness?

(A) Administration of antibiotics
(B) Advanced age
(C) Drinking unpasteurized milk
(D) Eating contaminated cold cuts
(E) Living in nursing home

49. A 15-day-old boy presents with conjunctivitis. Iodine staining bodies are seen in conjunctival scrapings. The most likely infectious form is a(n)

(A) elementary body
(B) reticulate body
(C) endospore
(D) exotoxin
(E) vegetative cell

50. A 45-year-old man presents to the emergency department with shortness of breath and a productive cough. His sputum was gelatinous and bloody. Gram stain of the sputum revealed numerous PMNs and gram-negative rods. Which of the following descriptions is most likely to fit the patient?

(A) Alcoholic
(B) Homeless
(C) Hiker
(D) IV drug user
(E) Veterinarian

51. An infant presents with fever, convulsions, and nuchal rigidity during the first month of life. Which of the following agents is the most likely cause?

(A) *Escherichia coli*
(B) *Haemophilus influenzae*
(C) *Listeria monocytogenes*
(D) *Streptococcus agalactiae*
(E) *Streptococcus pneumoniae*

52. A 60-year-old woman is hospitalized following a stroke and develops a high-grade fever with chills. She is catheterized due to urinary incontinence and receives cephalosporin for treatment of pneumonia. Blood cultures and Gram stain are performed by the laboratory. The organisms isolated are gram-positive cocci that are catalase-negative and capable of growth in 6.5% sodium chloride. Which of the following is the most likely causal agent?

(A) *Enterococcus faecalis*
(B) *Staphylococcus aureus*
(C) *Staphylococcus epidermidis*
(D) *Streptococcus pyogenes*
(E) Viridans streptococci

53. A 35-year-old man who is positive for HIV develops sepsis with the subsequent development of a necrotic lesion on the buttock that has a black center and an erythematous margin. Which of the following is the most likely causal agent?

(A) *Bacillus anthracis*
(B) *Clostridium perfringens*
(C) *Enterococcus faecalis*
(D) *Pseudomonas aeruginosa*
(E) *Staphylococcus aureus*

54. A 15-year-old girl develops a sore throat, fever, and earache of approximately one week duration. Upon examination by her physician, an erythematous rash is noted covering most of her body and her tongue appears bright red. Which of the following is the description of the causal agent?

(A) Gram-positive coccus, alpha hemolytic, catalase negative
(B) Gram-positive coccus, beta hemolytic, catalase negative
(C) Gram-positive coccus, alpha hemolytic, catalase positive
(D) Gram-positive coccus, beta hemolytic, catalase positive
(E) Gram-positive coccus, gamma hemolytic, catalase negative

55. A patient is admitted to the hospital because of a bleeding duodenal ulcer. Culture at 37.0 C (98.6 F) reveals a urease-positive, gram-negative, curved rod. Which of the following is a likely complication due to infection with the causal agent?

(A) Diarrhea
(B) Kidney stones
(C) Pseudomembranous colitis
(D) Stomach cancer
(E) Vomiting

56. Roommates of a 19-year-old college student become alarmed when he does not get up to go to swim practice in the morning and they are unable to wake him for his 11 AM class (he had complained of a headache and not feeling well the night before). The rescue squad finds a febrile, comatose young man with a petechial rash. In the emergency room, Kernig and Brudzinski signs are present. No papilledema is seen, so a spinal tap is done. Protein is high, glucose low. CSF WBC count is 9,000 (mainly PMNs) with few RBCs. The characteristics of the most likely causal agent are

(A) An enveloped dsDNA virus
(B) A naked (+)ssRNA virus
(C) A Gram-negative bacillus with a polyribitol capsule
(D) A Gram-negative, oxidase-positive diplococcus
(E) A Gram-positive, lancet-shaped, alpha-hemolytic diplococcus

MICROBIAL GENETICS/DRUG RESISTANCE

57. What type of genetic material is created by repeated transpositional recombination events?

(A) Chromosomal drug resistance genes
(B) Genetic operon
(C) Hfr chromosome
(D) Insertion sequences
(E) Multiple drug resistance plasmids

58. Which genetic material is found in pathogenic *Corynebacterium diphtheriae* but not in nonpathogenic normal flora diphtheroids?

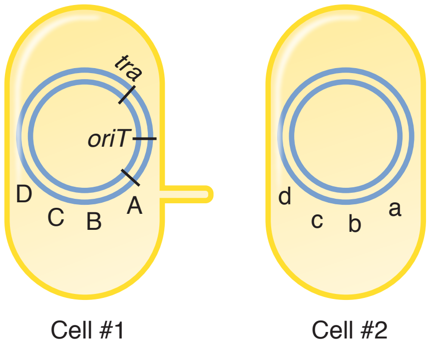
(A) A diphthamide on eEF-2
(B) An episome
(C) An F factor
(D) An integrated temperate phage
(E) Highly repetitive bacterial DNA

59. How is a prophage created?

(A) Through activation of the *recA* gene product of an exogenote
(B) Through infection of a bacterial cell with a virulent bacteriophage
(C) Through site-specific recombination of a temperate phage and bacterial DNA
(D) Through infection of a bacterial cell with lambda phage, lacking the lambda repressor
(E) Through excision of bacterial DNA and active lytic replication of a bacteriophage

60. If one cell of type one is mixed into a culture of 100 cells of type two, and culture conditions are optimized for conjugation BUT NOT for cell division, the cellular genotype that would predominate after overnight incubation would be that of

(A) Cell #1
(B) Cell #1 with new a, b, c, and d alleles
(C) Cell #2 with new A, B, C, and D alleles
(D) Cell #1 with a new a allele
(E) Cell #2 with a new A allele
(F) Cell #1 with new a and b alleles
(G) Cell #1 with new A and B alleles



61. Assume the following cells have no plasmids other than those mentioned. Which cell type would contain two molecules of DNA?

(A) F^+
(B) F^-
(C) Hfr

62. Assume the cells whose genotype is listed have no other plasmids than those indicated by the indicated genotype. Which bacterial cell is most likely to transfer chromosomal genes in linear order?

(A) F^+
(B) F^-
(C) Hfr

63. What bacterial gene transfer process is most sensitive to extracellular nucleases?

(A) Conjugation
(B) Generalized transduction
(C) Homologous recombination
(D) Site-specific recombination
(E) Specialized transduction
(F) Transformation

64. Following specialized transduction, if any of the bacterial genes transferred in are to be stabilized, what process must occur?

(A) Conjugation
(B) Generalized transduction
(C) Homologous recombination
(D) Site-specific recombination
(E) Specialized transduction
(F) Transformation

65. The ability of a cell to bind DNA to its surface and import it is required for which genetic process?

(A) Conjugation
(B) Generalized transduction
(C) Homologous recombination
(D) Site-specific recombination
(E) Specialized transduction
(F) Transformation

66. The process by which bacterial or plasmid DNA may be mistakenly incorporated (during assembly) into one phage being produced by the lytic life cycle and then that DNA-transferred to another bacterial cell which may acquire some new genetic traits is called

(A) Conjugation
(B) Generalized transduction
(C) Homologous recombination
(D) Site-specific recombination
(E) Specialized transduction
(F) Transformation

67. Recombination is required for stabilization of genetic material newly transferred by all of the following processes EXCEPT

(A) Movement of a transposon
(B) Integration of a temperate bacteriophage
(C) Transduction of a chromosomal gene
(D) Conjugal transfer of an R factor
(E) Transformation of a chromosomal gene

68. Lysogenic conversion

(A) is a change in pathogenicity due to the presence of a prophage.
(B) is the induction of a prophage to its virulent state.
(C) is the conversion of a virulent phage into a temperate phage.
(D) refers to the incorporation of a prophage into the chromosome.
(E) is the immunity that a prophage confers on a bacterium.

69. Which of the following events is most likely due to bacterial transformation?

(A) A formerly non-toxigenic strain of *Corynebacterium diphtheriae* becomes toxigenic.
(B) A non-encapsulated strain of *Streptococcus pneumoniae* acquires a gene for capsule formation from the extract of an encapsulated strain.
(C) A strain of *Neisseria gonorrhoeae* starts producing a plasmid-encoded β-lactamase similar to that another Gram-negative strain.
(D) A gene for gentamicin resistance from an *Escherichia coli* chromosome appears in the genome of a bacteriophage that has infected it.

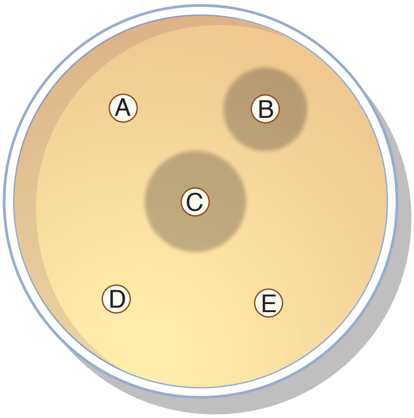
70. Which of the following mechanisms is most likely to be involved in multiple drug resistance transfer from one cell to another?

(A) Specialized transduction of a chromosomal gene for drug resistance
(B) Transformation of chromosomal genes
(C) Transposition
(D) Conjugation with a cell with a free plasmid carrying drug resistance
(E) Conjugation with a cell with chromosomal drug resistance

71. Which of the following agents, if introduced into a growing culture of bacteria, would halt growth but, if then removed, would allow growth to resume?

(A) Antiseptic
(B) Bacteriocide
(C) Bacteriostat
(D) Disinfectant
(E) Sterilizing Agent

72. A burn patient develops a purulent infection at the site of a skin graft. Culture of the pus is positive for *Pseudomonas aeruginosa*. The patient is started on anti-pseudomonal penicillin while a Kirby-Bauer agar disc diffusion test is requested for the isolate. The results are shown.



What is the correct interpretation of these lab results?

(A) The isolate is most sensitive to antibiotic B

(B) The isolate is most sensitive to antibiotic C

(C) The isolate is most sensitive to antibiotic E

(D) The isolate is resistant to antibiotic B

(E) Results cannot be analyzed without a key

73. A bacterial isolate from a patient with chronic sinusitis is shown to be sensitive to amoxicillin on a Kirby-Bauer agar disk diffusion test. A follow-up determination of the MIC of the drug is reported back from the laboratory at 2 μg/ml with an MBC of 1 μg/ml. What is the correct interpretation of this data?

(A) The drug is bacteriocidal.

(B) The drug is bacteriostatic.

(C) The drug should be administered to the patient at 1 μg/ml.

(D) The drug should be administered to the patient at 2 μg/ml.

(E) There has been a laboratory error.

MEDICALLY IMPORTANT VIRUSES

74. A 5-year-old girl presents to the pediatrician with complaints of a sore throat. Her mother also noticed that both of her eyes were slightly red. Examination reveals rhinopharyngitis with bilateral conjunctivitis. What activity likely led to the illness?

    (A) Hiking in a heavily wooded area
    (B) Eating undercooked shellfish
    (C) Playing with toys in a day care center
    (D) Traveling to a developing country
    (E) Swimming in a community pool

75. Serologic test results from a hepatitis patient reveal: anti-HBc positive, HBsAg positive, and anti-HBs negative. The correct interpretation of the patient's status is

    (A) No longer contagious
    (B) Immune to hepatitis B virus
    (C) Evidence of receiving hepatitis B vaccination
    (D) Hepatitis B virus chronic carrier state
    (E) Impossible to have both surface antigen and core antibody positive

76. A 6-year-old girl presents to the emergency department with a fever and a lacy body rash. Her mother says that yesterday the rash was only on her face, but by this morning, had spread to her trunk and extremities. Which of the following agents is most likely?

    (A) B19
    (B) HHV-6
    (C) Measles
    (D) Rubella
    (E) Varicella zoster virus

77. The best prospects for treatment and cure of microbial diseases are always those unique factors of a pathogen's life cycle that can be altered without affecting the survival of the host's own cells. In HIV, one such therapeutic target would be the products of the *pol* gene, which codes for the reverse transcriptase unique to the retroviral life cycle. If it were possible to ablate expression of the HIV *pol* gene, what other aspect of the virus's life cycle would be directly altered?

    (A) Transcription from proviral DNA
    (B) Production of viral mRNA
    (C) Integration of proviral DNA
    (D) Nucleocapsid
    (E) Viral maturation

78. A 28-year-old male ER resident was accidentally stuck with a needle from a hepatitis B virus-positive patient. He was too embarrassed to tell his attending of his mistake. Two months later, he began to feel fatigued and lost his appetite. When he ordered a hepatitis B serologic panel, he received the results as follows:

HBsAg	+
HBsAb	–
HBcAb	+
HBeAg	+
HBeAb	–

What is the status of the resident?

(A) Acute infection
(B) Chronic infection
(C) Fulminant infection
(D) Immune
(E) Uninfected

79. A prison inmate who was diagnosed with hepatitis six months ago is tested for his progress with the following results:

HBsAg	–
HBsAb	+
HBcAb	+
HBeAg	–
HBeAb	+

What is the status of the patient?

(A) Acute infection
(B) Chronic infection
(C) Fulminant infection
(D) Immune
(E) Uninfected

80. A 10-year-old boy is brought to the emergency department with a high fever, chills, headache, and nausea. He vomits at admission, where his temperature is 40.1 C (104.2 F), and he begins to hallucinate. A CT scan reveals encephalitis in one temporal lobe. Which of the following causal agents is most likely?

(A) California encephalitis
(B) Herpes simplex virus 1
(C) Polio virus
(D) St. Louis encephalitis
(E) West Nile virus

81. A 60-year-old woman who recently received a liver transplant develops a high fever and severe dyspnea with a dry hacking cough. Chest x-ray reveals bilateral interstitial infiltrates that are diffuse. Which of the following agents is most likely responsible for her condition?

(A) Adenovirus
(B) Cytomegalovirus
(C) Influenza virus
(D) Respiratory syncytial virus
(E) Rhinovirus

82. An 8-year-old boy from India was brought to the emergency department while visiting the U.S. because of a flaccid paralysis in his lower extremities. His mother explains that the child had a flu-like illness a couple of weeks earlier. How was the agent in the case likely acquired?

(A) Fecal-oral
(B) Mosquito
(C) Respiratory
(D) Sexual
(E) Tick

83. A 5-year-old girl presents with a fever and a generalized macular rash that is most dense on the scalp and trunk of the body. Several waves of lesions appear, one after another, and evolve rapidly into vesicles and then pustules over several days. The most likely disease and causal agent is

(A) Exanthem subitum due to cytomegalovirus
(B) Chickenpox due to the varicella-zoster virus
(C) Whitlow infection due to herpes simplex virus type 1
(D) Herpetic gingivostomatitis due to the varicella-zoster virus
(E) Infectious mononucleosis due to the Epstein-Barr virus

84. Infection of appropriate cells with a composite virus made up of Coxsackie virus capsid components and poliovirus RNA would yield progeny which would

(A) Have the host cell range of Coxsackie virus
(B) Also be composite viruses
(C) Show phenotypic mixing
(D) Have a recombinant genome consisting of both Coxsackie and poliovirus
(E) React with Sabin-vaccine–induced antibodies

85. An epidemic of nausea, vomiting, and watery diarrhea breaks out on shipboard during a cruise to the Virgin Islands. Which of the following accurately describes the most likely causal agent?

(A) Acid-fast oocysts
(B) Enveloped DNA virus
(C) Enveloped RNA virus
(D) Nonenveloped DNA virus
(E) Nonenveloped RNA virus

86. To design a vaccine against HIV infection, a logical goal would be to alter some native molecule or product of the virion in order to make it highly immunogenic. If you wished to prevent the attachment of the virus to helper T lymphocytes, which molecule or family of molecules might best be targeted?

(A) gp41
(B) gp120
(C) nucleocapsid protein
(D) p17
(E) p24

87. A woman in her late twenties presents to the emergency department disoriented and confused. She is unable to remember where she lived or even her phone number. She is admitted for observation and testing and begins to hallucinate and salivate excessively. On the east coast of the United States, what is the most common reservoir of this disease?

(A) Bat
(B) Cat
(C) Dog
(D) Fox
(E) Raccoon

88. An 11-month-old infant was brought to the emergency department with difficulty breathing and wheezing. History and physical examination reveal a slight fever, cough, and rhinorrhea that began about 2 days before. Analysis of the sputum reveals normal flora with the presence of giant multinucleated cells. Which of the following is the most likely cause?

(A) B19
(B) Influenza
(C) Parainfluenza
(D) Measles
(E) Respiratory syncytial virus

89. A 37-year-old executive for a local Health Maintenance Organization comes to your office because he has developed multiple blister-like lesions on his penis over the last 1–2 days. They are somewhat painful, and he is worried that he has AIDS. He denies homosexuality and intravenous drug abuse and had an HIV test prior to his marriage three years ago. He reports several similar episodes several years ago when he worked as a photographer in Nepal. He was never told what they were, and they resolved over several days without any treatment. His physical examination is remarkable only for the presence of 6–8 vesicular lesions 3–4 mm in diameter on the glans of the penis. There is no crusting, drainage, or bleeding. The lesions are moderately tender and there is mild inguinal adenopathy bilaterally. How does the causal agent produce its messenger RNA?

(A) By producing a positive sense intermediate
(B) By direct translation from the genome
(C) By transcription from proviral DNA
(D) By producing a negative sense intermediate
(E) By transcribing from the genomic DNA
(F) By reverse transcription from the genome
(G) By semi-conservative replication

90. Several individuals in the central United States from the ages of 5 to 25 have come down with symptoms of nausea, vomiting, and swelling of the parotid glands. Which of the following can be a complication of the disease?

(A) Guillain-Barré Syndrome
(B) Glomerulonephritis
(C) Orchitis
(D) Multiple sclerosis
(E) Reye syndrome

91. In the U.S., a baby has the greatest chance of acquiring which virus in utero?

(A) Cytomegalovirus
(B) Hepatitis B virus
(C) Herpes simplex virus
(D) Respiratory syncytial virus
(E) Rubella virus

92. Which of these viruses has RNA for both its genome and replicative intermediate?

(A) Cytomegalovirus
(B) Hepadnavirus
(C) Retroviruses
(D) Togaviruses
(E) Poxvirus

93. What is the most common lab testing method for diagnosing infectious mononucleosis?

(A) The Monospot test to detect EBV-specific antibody
(B) An assay for Epstein-Barr nuclear antigen
(C) The presence of atypical lymphocytes in the blood establishes the etiology
(D) A test for heterophile antibody, which cross-reacts with antigens found on a variety of animal red blood cells
(E) A simple procedure is done to isolate EBV from saliva, blood, or lymphoid tissue

94. What virus is noted for genetic reassortment, which leads to major pandemics about once every 10 to 11 years?

(A) Adenovirus
(B) Herpes virus
(C) Human immunodeficiency virus (HIV)
(D) Influenza virus
(E) Poliovirus

95. What virus is noted for such a high incidence of genetic drift that more than one antigenic variant can be isolated from most infected individuals who have high viral titers?

(A) Adenovirus
(B) Herpes virus
(C) Human immunodeficiency virus (HIV)
(D) Influenza virus
(E) Poliovirus

96. A 19-year-old male college student reports sore throat and extreme fatigue following even normal non-taxing tasks like getting dressed and going down to breakfast. He tells you that he has been sick for several weeks, that he has been feverish, and that his girlfriend now appears to be getting the same thing. His tonsils are inflamed with a white exudate adhering; cervical lymphadenopathy is prominent, as is splenomegaly. The most likely causal agent is

(A) ssDNA, naked icosahedral virus
(B) dsDNA, naked icosahedral virus
(C) dsDNA, enveloped complex virus
(D) dsDNA, enveloped icosahedral virus
(E) dsRNA, naked segmented virus
(F) –ssRNA, segmented, enveloped and helical virus
(G) –ssRNA, bullet-shaped, helical virus
(H) –ssRNA, naked, helical virus
(I) +ssRNA, naked, icosahedral virus
(J) +ssRNA, enveloped, icosahedral virus
(K) +ssRNA, enveloped, diploid virus

97. Cataracts and patent ductus arteriosus in a newborn suggest in utero infection with what viral family?

(A) Adenovirus
(B) Paramyxovirus
(C) Parvovirus
(D) Picornavirus
(E) Reovirus
(F) Togavirus

98. What is the primary means of spread for measles?

(A) Animal bite
(B) Fecal-oral
(C) Fomite spread
(D) Respiratory droplet spread
(E) Sexual contact
(F) Transfusion or intravenous drug abuse
(G) Tick bite

99. How are human papilloma virus type 4 warts spread?

(A) Animal bite
(B) Fecal-oral
(C) Fomite spread
(D) Respiratory droplet spread
(E) Sexual contact

100. A 15-year-old member of the high school swim team notices painless, umbilicated cutaneous lesions on the toes. Large eosinophilic cytoplasmic inclusions are present in the affected epithelia. What is the most likely causal agent?

(A) Adenovirus
(B) B19 virus
(C) Molluscum contagiosum virus
(D) Herpes simplex virus
(E) Human papilloma virus

101. A bone marrow transplant recipient becomes febrile and hypoxic and chest films demonstrate diffuse interstitial pneumonia. What is the most likely causal agent?

(A) BK virus
(B) Cytomegalovirus
(C) Herpes simplex virus
(D) Molluscum contagiosum virus
(E) Paramyxovirus
(F) Varicella-zoster virus

102. A 6-month-old infant presents with painless verrucous growths on the laryngeal folds. What is the most likely causal agent?

(A) B19 virus
(B) Cytomegalovirus
(C) Herpes simplex virus
(D) Human papilloma virus
(E) Molluscum contagiosum virus

MEDICALLY IMPORTANT FUNGI

103. An obese 32-year-old diabetic woman presents with complaint of red and painful skin in her abdominal skin folds. Examination reveals a creamy white material at the base of the fold. It is erythematous underneath and extends beyond the creamy material. Microscopic examination of the exudate reveals oval budding structures (3×6 µm) mixed with more budding elongated forms. The most likely causal agent is

(A) *Aspergillus fumigatus*
(B) *Candida albicans*
(C) *Epidermophyton floccosum*
(D) *Microsporum canis*
(E) *Sporothrix schenckii*

104. A 19-year-old migrant worker from the southwestern U.S. is brought to the family doctor complaining of cough, pleuritic chest pain, fever, and malaise. He also complains of a backache and headache. He is found to have an erythematous skin rash on his lower limbs. A chest radiograph reveals several calcifying lesions. Which of the following structures is most likely to be found?

(A) Broad-based budding yeast
(B) Monomorphic encapsulated yeast
(C) Nonseptate hyphae with broad angles
(D) Septate hyphae branching dichotomously at acute angles
(E) Spherules with endospores

105. An 18-year-old high school student in rural north Mississippi develops fever, cough, and chest pain. The cough, associated with weight loss, persisted. Because of poor performance at football practice, he was advised to see a physician. Lymph node biopsies stained with H and E reveal granulomatous inflammation and macrophages engorged with oval structures measuring 2–4 µm. Cultures incubated at room temperature grow powdery white colonies, which on microscopic study have tuberculate spores. The high school student most likely acquired the infection from which of the following?

(A) Desert sand
(B) Cat feces
(C) Soil enriched with bird excrement
(D) Another human via respiratory secretions
(E) Contaminated drinking water

106. A 32-year-old man from the southeastern U.S. is referred to a tertiary care center with chronic pneumonia. He also complains of malaise, weight loss, night sweats, chest pain, breathlessness, and hoarseness. Sputum smear revealed thick-walled, refractile, double-contoured yeast cells. What is the most common site of dissemination for the causal organism?

(A) Heart
(B) Liver
(C) Mucocutaneous
(D) Skin
(E) Spleen

107. A 55-year-old man who recently recovered uneventfully from a heart valve transplant presents to the emergency room with pleuritic chest pain, hemoptysis, fever, and chills. While he is being examined, he has a myocardial infarction and the medical team is unable to revive him. An autopsy revealed septate, acutely branching hyphae in many tissues. Which of the following organisms is most likely to be identified?

(A) *Aspergillus fumigatus*
(B) *Blastomyces dermatitidis*
(C) *Cryptococcus neoformans*
(D) *Histoplasma capsulatum*
(E) *Mucor* species

108. A 33-year-old HIV-positive man complains of headache and blurred vision. Physical examination reveals papilledema and ataxia. A head CT scan is normal, but CSF obtained by lumbar puncture reveals encapsulated organisms visible by India ink. Which of the following is true concerning this organism?

(A) It can also be seen as “spaghetti and meatballs” on KOH stain
(B) It consists of branching septate hyphae
(C) It exists as a mycelial form at room temperature and a yeast at 37.0 C (98.6 F)
(D) It is an encapsulated nondimorphic yeast found worldwide
(E) It is a nonencapsulated dimorphic yeast that reproduces by budding

109. A 32-year-old man who has AIDS presents to his physician with progressively increasing dyspnea over the past 3 weeks. He also complains of a dry, painful cough, fatigue, and low-grade fever. A chest x-ray reveals bilateral symmetrical interstitial and alveolar infiltration. Which of the following agents is the most likely cause?

(A) *Cryptococcus neoformans*
(B) *Cryptosporidium parvum*
(C) *Histoplasma capsulatum*
(D) *Pneumocystis jiroveci*
(E) *Toxoplasma gondii*

MEDICAL PARASITOLOGY

110. A 44-year-old woman returns home to New York after a 2-week camera safari to East Africa. She started chloroquine antimalarial prophylaxis 2 weeks prior to her departure for Kenya and continued throughout her foreign travel. She stopped taking the pills on her arrival home because they made her nauseated. Two weeks after her return, she develops paroxysmal fever and diaphoresis and is quickly hospitalized with febrile convulsions, jaundice, and anemia. Blood smears reveal red blood cells multiply infected with delicate ring-like trophozoites and rare sausage-shaped gametocytes. The stage of the parasite life cycle that is responsible for the appearance of the parasites 2 weeks after departure from the malarious area is the

(A) hypnozoite
(B) sporozoite
(C) exoerythrocytic schizont
(D) erythrocytic schizont
(E) merozoite

111. At a school nurse's request, a clinic in rural South Carolina sees a 9-year-old girl who appears listless and inattentive, although hearing and visual testing has been within normal limits. The physician finds the child thin, with the "potbelly" of malnutrition, and orders a fecal exam and CBC. The CBC reveals a microcytic, hypochromic anemia, and the fecal exam detects brown, oval nematode eggs approximately 65 microns in size, too numerous to count. What was the most likely means by which this child was infected?

(A) Ingestion of ova
(B) Ingestion of larvae
(C) Ingestion of cysts in muscle
(D) Skin penetration by larvae
(E) Mosquito transmission of sporozoites

112. An HIV-positive patient with a CD4+ count of 47 presents with diarrhea. Acid-fast structures are found in the stool. From this finding, which of the following is true?

(A) Infection is short lasting and self-resolving and requires no treatment
(B) If treated with antibiotics, the infection should resolve in 3–6 days
(C) Infection will resolve only with a combination of antituberculous drugs, and then it may take weeks
(D) Infection could have been prevented by avoiding cat feces and undercooked or raw meat
(E) Even with the best treatment, the infection may be unrelenting

113. A 24-year-old primiparous woman in her eighth month of gestation develops a positive IgM titer to *Toxoplasma gondii* for the first time. She should be advised by her physician that

(A) this child and all future fetuses are likely to be infected
(B) a newborn with a positive anti-*Toxoplasma* IgG response should be treated with anti-parasitics
(C) future infections can be avoided by proper vaccination and worming of cats
(D) retinochoroiditis can be prevented by drug treatment of an infant with a positive IgM response
(E) major organ damage can be reversed by prompt treatment of the newborn

114. A 35-year-old captain in the army reserves has been plagued by a painful, erosive lesion near his ear lobe since his return from Operation Desert Storm several years ago. He denies exposure to the toxic by-products of burning oil fields. Punch biopsy of the leading edge of the erosion reveals macrophages distended with oval amastigotes. How was this infection acquired?

(A) Contact with contaminated drinking water
(B) Bite of *Anopheles* mosquito
(C) Bite of reduviid bug
(D) Fecal contamination of food
(E) Direct human contact in barracks
(F) Bite of sandfly
(G) Bite of tsetse fly

115. A group of 6 college students undertake to climb Mt. Rainier outside Seattle on their spring break. They pack food and camping provisions except for water, which they obtain from the many fresh water mountain streams that arise at the summit. The adventure takes a little over a week to accomplish, and all return safely in good spirits to their classes the following week. Within the first week after their return, 5 of the 6 students report to the infirmary with profuse diarrhea and tenesmus. Each affected student experiences weakness and weight loss, and stool samples submitted to the lab are yellow, greasy, and foul smelling. What attribute of this parasite imparts its pathogenicity?

(A) Lytic enzymes
(B) Flagella
(C) Ventral sucking disc
(D) Encystment
(E) Toxic metabolites

116. After one week vacationing in Mexico, a 14-year-old girl presents with abdominal pain, nausea, bloody diarrhea, and fever. Stool specimens are collected and sent to the laboratory for bacteriologic and parasitologic examination. Bacterial cultures are negative for intestinal pathogens. The laboratory report reveals organisms with red blood cells inside them. The most likely causal agent is

(A) *Cryptosporidium parvum*
(B) *Entamoeba histolytica*
(C) *Giardia lamblia*
(D) *Toxoplasma gondii*
(E) *Shigella dysenteriae*

117. Four weeks after his arrival from Egypt, a 24-year-old graduate student presents with blood in his urine. Microscopic examination of his urine reveals the presence of eggs with terminal spines. In the interview he admits that he has been working on his family's rice field occasionally since his early childhood. The most likely etiologic agent of his complaint is

(A) *Entamoeba histolytica*
(B) *Fasciolopsis buski*
(C) *Schistosoma haematobium*
(D) *Schistosoma japonicum*
(E) *Schistosoma mansoni*

118. A 30-year-old woman presents to her gynecologist with complaints of vaginal itching and a frothy, yellow discharge. She also complains of painful urination. She admits to being sexually active with several men in the past two weeks. Cultures are negative for bacterial growth, but organisms are visible via a wet prep on low power. The most likely causal agent is

(A) *Candida albicans*
(B) *Trichophyton rubrum*
(C) *Chlamydia trachomatis*
(D) *Trichomonas vaginalis*
(E) *Giardia lamblia*

119. A 30-year-old missionary comes to the emergency department complaining of high fever, chills, severe headache, and confusion. He has recently returned from Africa. A peripheral blood smear reveals multiple ring structures and crescent-shaped gametes. Which of the following organisms is the most likely cause?

(A) *Leishmania* species
(B) *Plasmodium falciparum*
(C) *Plasmodium malariae*
(D) *Plasmodium ovale*
(E) *Plasmodium vivax*

120. A 3-year-old girl presents to her pediatrician with intense perianal itching. Her mother explains that the child has also been extremely irritable during the day and has not been sleeping well at night. Eggs with a flattened side were identified by the laboratory technician from a piece of scotch tape brought in by the parent. Infection with which of the following organisms is most likely?

(A) *Ascaris lumbricoides*
(B) *Echinococcus granulosus*
(C) *Entamoeba histolytica*
(D) *Enterobius vermicularis*
(E) *Trichuris trichiura*

121. A 12-year-old girl from Guatemala was brought to the emergency room with a prolapsed rectum. Examination of the rectum reveals small worms that resemble whips attached to the mucosa. A stool sample reveals eggs that are barrel shaped, with bipolar plugs. Which of the following is the most likely cause?

(A) *Ascaris lumbricoides*
(B) *Echinococcus granulosus*
(C) *Entamoeba histolytica*
(D) *Enterobius vermicularis*
(E) *Trichuris trichiura*

CLINICAL INFECTIOUS DISEASE

Case Histories

Case A: A 28-year-old known alcoholic man presents with fever and productive cough. He was basically well until 3 days ago when he noticed perspiration, cough, shaking chills, and headache. His cough has been associated with the production of a yellowish-green sputum, which occasionally was tinged with brownish streaks, but was not foul smelling. A Gram stain shows Gram-positive cocci in pairs and short chains.

A. What laboratory tests could you use to identify the genus?

Answer: Catalase test (negative) to genus.

B. When plated on blood agar, what other bacterium might you isolate and confuse the causal agent with, and why? What test(s) could distinguish the two?

Answer: Viridans strep; optochin and bile.

C. What procedure would you perform to type the isolate?

Answer: Quellung reaction with known antibodies to capsule (not antibodies to cell-wall antigens).

Case B: The patient in Case A developed meningitis and died.

A. What would be the expected CSF cell count?

Answer: High.

B. What would be the expected CSF protein and sugar values?

Answer: Protein high, sugar low.

Case C: A 46-year-old, HIV-positive man complains of malaise, weight loss, fever, and night sweats of 6 weeks duration. More recently, he has developed a cough productive of bloody sputum. Physical exam reveals bronchial breath sounds with crepitant rales over the right upper chest. His CD4 cell count is 560 cells/mm^3. Auramine-rhodamine stain of the sputum is positive, and a chest radiograph reveals hilar lymphadenopathy with a small cavity and streaky infiltrates in the upper lobe.

A. What attribute of the most likely causal agent promotes its survival in reticuloendothelial cells?

Answer: Sulfatides are sulfolipids which hydrolyze to make sulfuric acid. They impede the fusion of lysosomes with the phagosome.

B. What attribute of the causal agent is injected in order to elicit a positive skin test?

Answer: Tuberculin (outermost protein) plus mycolic acids (long-chain fatty acids in the envelope)

C. What immune cells are most important in the response to this agent?

Answer: Th1 cells and macrophages (granuloma formation)

Case D: A patient presents with multiple, crusted and oozing, honey-colored lesions.

A. What is the skin infection?

Answer: Impetigo.

B. What two bacteria would you expect to isolate on culture?

Answer: Streptococcus pyogenes *(often honey-colored crusted) and/or* Staphylococcus aureus *(often longer-lasting vesicular or with bullae).*

C. How would you separate the two?

Answer: Catalase test positive for Staphylococcus, *negative for* Streptococcus.

Case E: A patient had intermittent bouts of general malaise, fever with weight loss, and progressive anemia. She presents also with a heart murmur.

A. What additional physical sign might occur and what causes it?

Answer: Splinter hemorrhages are caused as septic emboli are thrown from heart valves. They are also seen in trichinosis and trauma.

B. What is her underlying condition and what are the most commonly involved bacteria?

Answer: Damaged heart valve;
Viridans streptococci (associated with bad oral hygiene or dental work) or
Enterococcus faecalis *or* E. faecium *if she has had bowel surgery.*

C. How would you distinguish the colony on blood agar?

Answer: Alpha hemolytic not inhibited by optochin; a viridans streptococcus.

Case F: A family of Christian Scientists brings their youngest child to the emergency room because of fever and a stiff neck. The 18-month-old child is acutely ill with a temperature of 40.0 C (104.0 F). CSF is Gram stained, examined in a rapid test, and also cultured. A Gram stain shows pleomorphic, gram-negative rods.

A. What laboratory test could confirm the identity of the isolate?

Answer: Meningitis screen, a series of immunologic rapid identification tests (usually EIAs using known antibodies), followed by growth of CSF sediment or filtrate on special media and drug susceptibilities.

B. What growth factors are required to grow the isolate on blood agar?

Answer: X = hemin and V = NAD.
Chocolate agar provides both X and V.

C. What is the drug of choice?

Answer: Cefotaxime or ceftriaxone.

D. What is the mechanism of action of the vaccine which would have prevented this condition?

Answer: It is a conjugated vaccine containing the polyribitol phosphate capsular material of the most important serotype (the hapten) covalently coupled to the diphtheria toxoid (protein carrier). The hapten stimulates the B lymphocyte, the carrier stimulates the Th cell, and together, isotype switching becomes possible so that something other than IgM is made.

Case G: A 23-year-old woman presents with lower back pain, fever, and dysuria of 3 days' duration. Urinalysis reveals many white blood cells (WBC) and WBC casts. Gram stain of the uncentrifuged urine reveals numerous Gram-negative bacilli per oil immersion field. On culture, extremely motile bacteria form waves of confluent growth.

A. What is the most important biochemical characteristic of this organism? Why?

Answer: Proteus, *urease-producing Enterobacteriaceae; kidney stones induced.*

Case H: A child developed a unilateral mucopurulent conjunctivitis 10 days after birth. A conjunctival specimen was sent to the laboratory and inoculated into tissue culture cells. Iodine-staining inclusion bodies were produced.

A. What is unusual about the chemical makeup of the organism?

Answer: ATP defective mutant, also muramic acid missing from peptidoglycan.

B. What are the two forms of the organism?

Answer: Elementary bodies (extracellular) and reticulate bodies = replicating forms.

C. What would you see on Gram stain?

Answer: Nothing in the cells—poorly Gram staining.

D. What serologic type caused the child's problem?

Answer: If U.S. kid, serotypes D-K.

Case I: A 27-year-old attorney is hospitalized. He was in excellent health until two days earlier when he noted malaise, fatigability, and profound anorexia. He remembers approximately 6–8 weeks ago receiving a tattoo while vacationing in the Caribbean.

A. How would you confirm your clinical diagnosis?

Answer: HBsAg and IgM to HbcAg.

B. What is meant by the "window"?

Answer: A time period between the end of the detectable presence of HBsAg and the beginning of the production of HBsAb. HBcAb and HBeAb are present.

C. What antigen's persistence beyond 6 months post-infection is indicative that the patient is entering a carrier state?

Answer: HBsAg past 6 months.

D. What antigen correlates with viral production?

Answer: HBeAg.

E. Does the virus carry a virion associated polymerase? If so, what kind?

Answer: Yes, RNA-dependent DNA polymerase (Hepatitis B replicates through an RNA intermediate).

Case J: A young man became ill with a sore throat and swollen tonsils, marked fatigue, cervical adenopathy, a palpable spleen, and a pruritic erythematous rash that started after self-administration of ampicillin.

A. What is the most likely disease? What are the most common laboratory diagnostic tests? What does the antibody test measure?

Answer: Infectious mononucleosis; monospot test (measures heterophile antibody which is not specific to EBV antigen) plus CBC.

B. What type of cells are the Downey type II cells?

Answer: T lymphocytes. (Reactive cells, not infected.)

C. What cells does the virus infect? Through what receptor does the lymphocytic infection begin?

Answer: EBV infects epithelial cells and B lymphocytes, whose receptor is CD21 = CR2.

Case K: A 35-year-old woman presents with a unilateral vesicular rash.

A. The most likely diagnosis is

Answer: Shingles.

B. Describe the virion's nucleic acid.

Answer: Linear dsDNA.

C. Patient had a previous history of what other disease?

Answer: Chickenpox.

Case L: A 27-year-old man presents to the hospital emergency room with a cough, chest pain, and fever. Two days before admission he developed a nonproductive cough. Rales are heard. Gram stain of sputum was negative. Sputum cultures on blood agar were also negative. Culture on a special medium containing cholesterol, purines, and pyrimidines produced colonies in 10 days. Serology 3 weeks later (when he returned because of persistent cough but feeling better) showed cold agglutinins.

A. What is the probable causal agent?

Answer: Mycoplasma pneumoniae.

B. Why did the organism not show up on the Gram stain?

Answer: Organism does not have a cell wall and does not stain with either the primary or counterstain in the Gram stain.

C. What antibiotics do you NOT use?

Answer: Penicillin/cephalosporin.

Case M: A 65-year-old retired male police officer reports to an emergent care facility complaining of fever, sore throat, shortness of breath, dry cough, and generalized muscle aches and pains. On examination the patient is pale, tachycardic, and tachypneic. His conjunctivae are congested and rales and wheezes are heard over both lung fields. A chest radiograph shows diffuse bilateral infiltrates and a hemagglutination inhibition antibody test is positive at high titer.

A. What is your diagnosis?

Answer: Influenza

B. What drugs are available to treat this disease?

Answer: Amantadine/rimantadine (inhibit uncoating)
Zanamivir/oseltamivir (inhibit neuraminidase)

C. To what viral family does it belong?

Answer: Orthomyxovirus

D. Where in the cell does it replicate?

Answer: Cytoplasm and nucleus

E. What vaccine might have prevented this?

Answer: Killed, H3N2, H1N1 plus one strain of Influenza B

F. What attribute of the agent causes pandemics?

Answer: Segmented genome can be reassorted, causing genetic shift.

Case N: A 4-year-old boy is brought to the pediatrician by his mother, who is concerned by his lack of appetite and loss of weight. He has had diarrhea fairly constantly over the preceding two-week period, which occasionally has been associated with vomiting. On examination, the child is in the 60% percentile of weight for his age and has mild epigastric tenderness. A fresh stool sample, collected rectally, is yellow, greasy and malodorous and contains motile organisms.

A. To what taxonomic group does this causal agent belong?

Answer: (Giardia lamblia) Flagellated protozoan

B. How was this child infected?

Answer: Fecal/oral contamination with cysts

C. What is the mechanism of pathogenesis?

Answer: Organism adheres in the upper duodenum using a ventral sucking disk. This blocks common bile ducts, causes fat malabsorption and steatorrhea

Case O: A 35-year-old worker at a plant nursery seeks his physician for a suppurative lesion on one of his fingers. A smear is taken of the drainage and stained. Cigar-shaped yeasts are detected.

A. What is the causal agent?

Answer: Sporothrix schenckii.

B. Is the fungus dimorphic or monomorphic?

Answer: This is DIMORPHIC FUNGUS consistent with Sporothrix. You can tell from cigar-shaped yeast (in tissues generally tough to visualize) and hyphae with sleeve and rosettes arrangement of conidia in culture.

C. Treatment

Answer: Itraconazole but oral KI given in milk will also clear up.

Case P: A Christian missionary returns to the United States from Central America with high fever, chills, headache, and confusion. On examination his temperature is 39°C and he is pale and tachycardic. Both liver and spleen are enlarged and tender to palpation. Laboratory tests reveal microcytic anemia, thrombocytopenia, hyperbilirubinemia, and hypoglycemia, and a blood film is examined.

A. What is the diagnosis?

Answer: Plasmodium vivax *malaria*

B. What is the treatment?

Answer: Chloroquine, quinine, amodiaquine, fansidar, halofantrine etc. (Lots of drug resistance is now occurring)

C. Is primaquine required? Why or why not?

Answer: Yes, to prevent relapse and kill hypnozoites. This is called "radical cure" and it is necessary for P. vivax *and* P. ovale *malarias.*

D. How did the patient acquire this disease?

Answer: Bite of female Anopheles *mosquito injects sporozoites.*

Case Q: A 50-year-old Missouri farmer was referred to the hospital because of malaise, weakness, weight loss, fever, and a palpable spleen. Examination of the mouth reveals a painless ulcerated lesion. A punch biopsy of the lesion is obtained and submitted for laboratory study. Histologic study revealed oval structures measuring 2–5 μm, packing the macrophages.

A. What is the most likely causal agent, and what are the distinctive forms?

Answer: Histoplasma capsulatum *with the intracellular oval yeasts and the tuberculate macroconidia (and microconidia) in the hyphal state.*

B. Where in nature will you find the fungus in large numbers?

Answer: Great central riverbed plains. Chicken coops in Missouri 100% infected. Indianapolis has had an ongoing outbreak and has major problems with it disseminating in their AIDS patients; NY City also high.

Case R: A 75-year-old woman who has suffered chronic otitis media, is brought to the hospital by the staff of her long-term care facility. She has complained of dizziness and drowsiness, and preliminary examination reveals signs of meningismus. A CT scan is negative for parenchymal lesions of the brain, although the mastoid cavities are inflamed. A lumbar puncture reveals 2130 leukocytes/μL, 1.55 mm glucose/L (concomitant blood sugar 2.6 mmol/L), and 1582 mg protein/L. Gram staining organisms are absent, but filamentous forms are cultured.

A. What is your diagnosis?

Answer: Aspergillus fumigatus *meningitis*

B. How would you describe this organism?

Answer: it is a monomorphic, dematiaceous fungus

C. What is the treatment of choice?

Answer: Amphotericin B or itraconazole

Case S: A 34-year-old accountant presents to the emergency room because of headache and fever of 3 days' duration. The day before admission his wife noted mild confusion and irritability. Lumbar puncture revealed an opening pressure of 300 mm, 200 red blood cells, 90% of the WBCs which are lymphocytes, sugar of 85 mg/dL (concomitant blood sugar of 110 mg/dL), and protein of 65 mg/dL. Bacteriologic smears (and ultimately also the bacterial cultures) were negative, as were India ink preparations. All latex particle agglutination tests for fungal and bacterial capsules done on the patient's CSF were also negative. The patient's condition did not improve despite appropriate therapy, and he died 10 days after hospitalization.

A. What is the most likely diagnosis?

Answer: Herpes simplex encephalitis

B. What is the virus's shape?

Answer: Icosahedral with nuclear membrane envelope.

C. Where within the cell does the virus replicate?

Answer: Nucleus for both DNA synthesis and assembly.

D. What other members belong to the same family?

Answers: EBV, Varicella-Zoster, Cytomegalovirus.

Case T: A 20-year-old man presents to the emergency department complaining of profuse bloody diarrhea of two days duration. On examination he has a purpuric rash over a large portion of his body, although his temperature is normal. The patient is dehydrated and weak, and lab values reveal an elevated blood urea nitrogen and creatinine, with thrombocytopenia. PT and PTT are within normal limits. Culture of the feces grew organisms which produced both colorless and colored colonies on sorbitol MacConkey medium.

A. What is your diagnosis?

Answer: Enterohemorrhagic Escherichia coli, (EHEC)

B. What is the most likely source of his infection?

Answer: Hamburger, fecal contamination from bovine feces

C. What is the most likely serotype in the United States?

Answer: O157:H7

D. What is the mechanism of pathogenesis?

Answer: Toxin (verotoxin) is Shiga-like and inhibits the 60S ribosomal subunit, thereby stopping eukaryotic protein synthesis.

Case U: A patient presents with anogenital warts.

A. What is the virus that probably caused the tumors?

Answer: Human papilloma virus.

B. What serotypes are most commonly associated with this clinical presentation?

Answer: 6 and 11

C. Are they premalignant?

Answer: Rarely.

D. What serotypes are most commonly associated with cervical intraepithelial neoplasia?

Answer: 16, 18, and 31. These are sexually transmitted.

E. What type of vaccine is now available that could have prevented this infection?

Answer: 4 serotypes of capsid protein created by recombinant DNA technology.

Case V: A girl received a bone marrow transplant for the treatment of leukemia. Nine weeks after the transplant her temperature rose, she became dyspneic and died. Impression smears were taken from the cut surface of the lower lobe of the left lung. The smears were stained with H&E. Intranuclear inclusions with perinuclear clearing were found.

A. Why did the patient develop pneumonia?

Answer: Immunocompromised—No T cells.

B. How would you describe what you would see (using only two words)?

Answer: Owl's eyes: cells with prominent basophilic intranuclear inclusion bodies.

C. What is the virion's nucleic acid type? To what viral family does it belong?

Answer: dsDNA; Herpes viruses.

Case W: A young woman developed a feverish illness with painful swelling of her knee, elbow, and wrist joints. She has a sparse rash on the distal parts of her limbs, consisting of small hemorrhagic pustules with an erythematous base. A smear was obtained from the exudate of the exanthem and Gram stained. The stain showed intracellular gram-negative diplococci.

A. What disease does she have?

Answer: Disseminated gonococcal infection.

B. What is the major mechanism of pathogenesis?

Answer: Pili, for attachment to epithelial surfaces—colonizing factor along with outer membrane proteins. Also exhibit antigenic variation and protect from phagocytosis.

ANSWERS AND EXPLANATIONS

1. **Answer: C.** Gram-positive cocci (alpha hemolytic streptococci) and gram-negative cocci (*Neisseriae*) are normally present in the throat. There is no way to differentiate pathogens from non-pathogens by the Gram stain.

2. **Answer: E.** The cephalosporin that inhibits prokaryotic cell peptidoglycan cross linkage will not likely be effective against the complex carbohydrate cell wall. Isoniazid, which appears to inhibit mycolic acid synthesis, also would not likely work. Metronidazole would not work on an aerobic organism. Shiga toxin is only effective against eukaryotic ribosomes. Tetracycline (the correct answer) would have the greatest chance of success. However, it may not be taken up by the cell, or the cell could have an effective pump mechanism to get rid of it quickly.

3. **Answer: C.** Mitochondria are found only in eukaryotic organisms so both viruses and bacteria lack them.

4. **Answer: A.** The clue of a complex carbohydrate cell wall (chitin, glucan or mannan) defines the organism as a fungus. The mention that the organism was gram positive was a tricky clue, because of course, the gram stain is used diagnostically to differentiate between the two major categories of bacteria (prokaryotes; **choice D**). The student should remember that some fungi will stain gram positive, however, because their thick cell wall makes them retain the gram stain just as a gram positive bacterium would. Parasites (**choice B**) do not possess a cell wall, prions (**choice C**) are infectious proteins, prokaryotes (**choice D**) have a peptidoglycan cell wall, and viruses (**choice E**) are acellular.

5. **Answer: E.** The attribute of microorganisms which associates most strongly with the causation of granulomas is the fact that they live intracellularly. This causes stimulation of the Th1 arm of the immune response, and the production of the cytokines of cell-mediated immunity, with the net result of the formation of granulomas in the infected tissues. Some organisms which are extracellular will also produce granulomas, but in those cases it is generally the chronic persistence and indigestibility of the pathogen which cause that result. Lipopolysaccharide (**choice A**) is a synonym for endotoxin, which causes gram negative shock, but not granuloma formation. Pili (**choice B**) are surface structures of some bacteria which mediate attachment to cellular surfaces. Exotoxins (**choice C**) are secreted toxins which may cause cell damage in a number of ways, and superantigens (**choice D**) cause stimulation of large numbers of clones of T lymphocytes and macrophages to cause symptoms similar to endotoxin shock.

6. **Answer: A.** Catheters, shunts and prosthetic devices which are left in the body long-term, are almost always coated with Teflon which is extremely slippery. Organisms which are capable of adherence to Teflon (or the enamel of teeth), do so by creation of a biofilm, which allows them to change the surface tension of the liquid around them and thereby "glue" themselves to the material. Ergosterol (**choice B**) is the major sterol in the cell wall of fungi, and is important in membrane integrity, but not adherence. Peptidoglycan (**choice C**) is the cell wall material of bacteria, and is responsible for the shape of bacteria, but not their adherence. IgA proteases (**choice D**) can assist in

the adherence of bacteria to mucosal surfaces but would not be important in adherence to an intravenous catheter, and although pili (**choice E**) mediate attachment of bacteria to human cells, they would not be important in adherence to Teflon.

7. **Answer: A.** Botulinum toxin (in Botox) inhibits release of acetylcholine and results in a flaccid paralysis. Inhibition of glycine and GABA (**choice B**) describes the action of Tetanus toxin which causes a rigid paralysis. The toxin of *Clostridium perfringens* is a lecithinase (**choice C**) which directly disrupts cell membranes. Toxic shock syndrome toxin-1 and the pyrogenic exotoxins of *Streptococcus pyogenes* act as superantigens (**choice D**) which cause systemic inflammatory response syndrome. Ribosylation of eukaryotic elongation factor-2 (**choice E**) is the mechanism of action of the diphtheria toxin and *Pseudomonas* exotoxin A. Ribosylation of Gs (**choice F**) is the mechanism of action of the cholera toxin and the labile toxin of Enterotoxigenic *Escherichia coli*.

8. **Answer: A.** The easiest way to differentiate between *Staphylococcus* and *Streptococcus* is the catalase test (**choice A**). This is important because they can have similar presentations. Coagulase (**choice B**) differentiates between members of the genus *Staphylococcus*. Hemolysis pattern (**choice D**) is inconclusive. Growth of organism in sodium chloride (**choice C**) can be useful for *Enterococcus*. PCR (**choice E**) is currently used to identify organisms that are difficult to culture.

9. **Answer: A.** Atherosclerosis leads to poor circulation to the lower extremities, which in turn lowers the oxidation-reduction potential of the tissues. All this predisposes to infections caused by anaerobic microorganisms, in this case, *Bacteroides* and streptococci. The patient is suffering from anaerobic cellulitis or possibly myonecrosis.

10. **Answer: E.** Partially acid-fast branching rods in a patient with lobar pneumonia suggests *Nocardia*. All the other agents listed are acid-fast bacilli, not branching rods.

11. **Answer: D**. The causal agent is *Legionella pneumophila*. The clues are dry cough, smoking, weakly gram-negative, and growth on buffered charcoal yeast agar. Remember that *Legionella* is one of the 4 sisters **ELLA** that worship in the **cysteine** chapel. Other sisters include *Francisella*, *Brucella,* and *Pasteurella*. A capsule (**choice A**) would identify agents such as *Streptococcus pneumoniae*. No cell wall (**choice B**) describes *Mycoplasma*. Optochin-sensitive (**choice C**) also describes *Streptococcus pneumoniae*. Serpentine growth in vitro (**choice E**) describes *Mycobacterium tuberculosis*.

12. **Answer: C**. The causal agent is *Borrelia burgdorferi*, and the disease is known as Lyme disease. The clues are facial palsy, rash, fever and malaise. *Borrelia* is spread by ticks.

13. **Answer: B**. The causal agent is *Yersinia pestis*. The clues are high fever, swelling in the armpits and groin, and gram-negative rods with bipolar staining. *Yersinia pestis* is endemic in the U.S. in the desert southwest.

14. **Answer: B.** Infant botulism is an infection started by the ingestion of *Clostridium botulinum* endospores from the environment. The spores germinate in the alkaline pH of the immature gastrointestinal tract and the toxin is produced *in vivo*. In adult botulism, the preformed toxin is ingested.

15. **Answer: D.** The reservoir for *S. enterica* subsp. *typhi* is humans. Other subspecies of *Salmonella* have animals as their reservoirs.

16. **Answer: C.** Each cell divides into two at each generation following the single lag phase. So at the end of the first 10 minutes there are still 5×10^2, and then at the end of the first 20 minutes (total) there are 10×10^2. At the end of 30 minutes total time there will be 20×10^2, and at the end of the total time, 40×10^2, which is written 4×10^3 in proper scientific notation.

17. **Answer: C.** The description strongly suggests that she has myonecrosis. Therefore, the causal agent (at least one) is *C. perfringens. C. perfringens* is an anaerobe; therefore **choice A** is wrong. *Clostridia* are all gram-positive; therefore, **choice B** is wrong. *C. perfringens* produces concentric areas of hemolysis; therefore, **choice D** is wrong. *C. perfringens* is a marked lecithinase producer; therefore, **choice C** is correct.

18. **Answer: D.** The patient has "scalded skin" syndrome caused by *S. aureus*. The genus *Staphylococcus* would be distinguished from *Streptococcus* by staphylococcal production of catalase. But the species (*S. aureus*) would be distinguished from *S. epidermidis* on the basis of *S. aureus* production of coagulase. Bacitracin sensitivity is characteristic of *Streptococcus pyogenes*, and bile solubility is characteristic of *Streptococcus pneumoniae*.

19. **Answer: A.** The clue is gram-negative curved rods with polar flagella often in pairs to give a "seagull" appearance, microaerophilic on special media and growing at 42°C. That description is most compatible with *Campylobacter jejuni*. Poultry are the most important reservoirs, so **choice A** is the correct response.

20. **Answer: A.** The causal agent is *Neisseria meningitidis*. The clues are age, dorm room, petechial rash, and nuchal rigidity. *Neisseria meningitidis* is a gram-negative diplococcus and can ferment maltose. Remember that *Neisseria* ***m****eningitidis* can ferment **m**altose. Gram-negative coccus, ferments glucose only (**choice B**) describes *Neisseria gonorrhoeae.* Gram-negative coccobacillus, capsular serotype b (**choice C**) describes *Haemophilus influenzae*. Gram-positive coccus, alpha hemolytic, optochin-sensitive (**choice D**) describes *Streptococcus pneumoniae.* Gram-positive rods, growth at 4°C (**choice E**) describes *Listeria monocytogenes.*

21. **Answer: D.** The clues are endocarditis, heart valve replacement, and gram-positive cocci that are catalase-positive and coagulase-negative. Many times *Staphylococcus epidermidis* can be a contaminant, but the fact that it was present in 3 consecutive blood cultures identifies it as the causal agent. *Enterococcus faecalis* (**choice A**) is catalase-negative. *Kingella kingae* (**choice B**) is a gram-negative rod. *Staphylococcus aureus* (**choice C**) is coagulase-positive. *Staphylococcus saprophyticus* (**choice E**) is the causal agent of UTIs, not endocarditis.

22. **Answer: E.** Capsules, cell wall, and cytoplasmic membranes are found in both gram-positive and gram-negative bacteria. Endospores (**choice D**) occur with certain gram-positive bacteria, e.g., *Bacillus* and *Clostridium*. Only gram-negatives have an outer membrane.

23. **Answer: D**. The clues are travel, constipation, which is more common than diarrhea, enlarged spleen, and rose-colored spots on the abdomen. It also is an H_2S producer and motile. Remember, salmon(ella) swim upstream (are motile). EHEC (**choice A**) does not produce H_2S and produces bloody diarrhea. ETEC (**choice B**) does not produce H_2S and produces watery diarrhea. *Salmonella enterica* subsp. *enteritidis* (**choice C**) diarrhea (watery, can be bloody) is associated with consumption of raw or undercooked poultry. *Shigella dysenteriae* (**choice E**) does not produce H_2S and causes bloody diarrhea.

24. **Answer: E.** The disease here is whooping cough, caused by *Bordetella pertussis*. The pertussis toxin (also known as the lymphocytosis-promoting toxin) is not believed to be directly cytotoxic, but stimulates adenylate cyclase by ribosylating regulatory proteins. It causes a variety of effects depending on the cell type involved: insulin secretion, lymphocytosis, and alteration of immune effector cells. Of the distractors: the filamentous hemagglutinin (**choice F**) mediates attachment; the adenylate cyclase toxin (**choice B**) stimulates local edema; the organism produces only a small zone of hemolysis around its colonies, so **choice C** is not true; it is an aerobe and does not grow anaerobically (**choice D**). All systemic manifestations of the disease arise from the circulation of the toxins, not the organism itself (**choice A**).

25. **Answer: B.** In children younger than age 5, the most serious complication of EHEC is hemolytic uremic syndrome, or HUS. This is because the toxin (which inhibits protein synthesis) can also bind to the glomerular epithelial cells. Also, because this toxin is Shiga-like, it is important to remember that *Shigella* can also lead to HUS.

26. **Answer: A.** The causal agent is *Streptococcus pneumoniae*. It is a gram-positive coccus, which is alpha hemolytic and optochin-sensitive, and it is the most common cause of pneumonia in the elderly. It is the capsule of *S. pneumoniae* which protects it against phagocytosis. That is why asplenic individuals have a difficult time clearing infections with *S. pneumoniae,* because a major antibody-producing organ is missing and phagocytosis is inhibited in organisms with capsules. Coagulase (**choice C**) is an anti-phagocytic attribute of *Staphylococcus aureus*. M protein (**choice D**) of *Streptococcus pyogenes* is anti-phagocytic. Catalase (**choice B**) breaks down H_2O_2, and teichoic acids (**choice E**) mediate adherence. Neither protects against phagocytosis.

27. **Answer: B.** The causal agent *E. coli* is gram-negative, and the primary means of developing septic shock with gram-negative organisms is via lipopolysaccharide (endotoxin).

28. **Answer: C.** The causal agent is *Rickettsia rickettsiae*, the disease is Rocky Mountain spotted fever. The clues are North Carolina (located in the tick belt of U.S.), rash that spread from extremities to trunk, conjunctivitis, and proteinuria. Rocky Mountain spotted fever is spread via a tick vector.

29. **Answer: B.** The case diagnosis is poststreptococcal glomerulonephritis caused by group A strep. The Lancefield group is determined by the C carbohydrate and the serotype is determined via the M proteins. This is particularly important in post streptococcal glomerulonephritis, as certain strains, such as the M12 serotype, are more commonly associated with this type of nonsuppurative sequela.

30. **Answer: B.** The causal agent is *Bordetella pertussis*. The clues are severe cough, can't catch breath, vomiting, incomplete vaccination history, conjunctival redness, and gram-negative coccobacilli on Bordet-Gengou medium. Pertussis toxin works by ADP ribosylation of G_i. ADP ribosylation of elongation factor 2 (**choice A**) describes toxins found in *Corynebacterium diphtheriae* and *Pseudomonas aeruginosa*. ADP ribosylation of a GTP-binding protein (**choice C**) describes the toxins found in ETEC and *Vibrio cholerae*. Blocks release of acetylcholine (**choice D**) describes the toxin found in *Clostridium botulinum*. Blocks release of inhibitory transmitters GABA and glycine (**choice E**) describes the toxin found in *Clostridium tetani*.

31. **Answer: D.** The toxin described is tetanus toxin, which would be acquired via some type of penetrating wound. Eating home-canned foods (**choice A**) describes transmission of adult botulism. Fecal-oral, travel to foreign country (**choice B**) describes ETEC, *Vibrio cholerae*, etc. Infant given honey during the first year of life (**choice C**) describes infant botulism. Respiratory, with incomplete vaccination history (**choice E**) describes *Bordetella pertussis*, for example.

32. **Answer: D.** The diagnosis is infant botulism caused by *Clostridium botulinum*. The clues are flaccid paralysis, constipation, difficulty breathing, tox screen of stool, and age. The flaccid paralysis is due to a toxin that blocks the release of acetylcholine. ADP ribosylation of eukaryotic elongation factor 2 (eEF-2; **choice A**) describes toxins found in *Corynebacterium diphtheriae* and *Pseudomonas aeruginosa.* ADP ribosylation of G_i, an inhibitory subunit of the G protein (**choice B**), describes *Bordetella pertussis.* ADP ribosylation of GTP-binding protein (**choice C**) describes the toxins found in ETEC and *Vibrio cholerae.* Blocks release of inhibitory transmitters GABA and glycine (**choice E**) describes the toxin found in *Clostridium tetani.*

33. **Answer: C.** The causal agent is *Corynebacterium diphtheriae*. The clues are incomplete vaccination history and (dirty gray pseudomembrane which causes bleeding when displaced). *C. diphtheriae* are gram-positive rods, and the toxin functions by inhibition of protein synthesis.

34. **Answer: A.** The causal agent is *Vibrio cholerae*. The clues are history of travel and voluminous "rice water" diarrhea. *Vibrio* are gram-negative curved rods, and the toxin functions by increasing intracellular cAMP.

35. **Answer: A.** The causal agent is *Helicobacter pylori*. The clues are midepigastric pain, relief after meals, positive urease test. *H. pylori* is a gram-negative curved rod that is microaerophilic. Gram-negative rod, aerobic (**choice B**) describes *Pseudomonas aeruginosa*, for example. Gram-negative rod, facultative anaerobe (**choice C**) describes *Escherichia coli*, for example. Gram-positive rod, aerobic (**choice D**) describes *Bacillus,* for example. Gram-positive rod, microaerophilic (**choice E**) does not describe a medically relevant genus.

36. **Answer: B.** The causal agent is *Streptococcus pyogenes*. The clues: sore throat, beta hemolytic, gram-positive cocci. To answer this question, you have to know that the agent is gram-positive and that gram-positive organisms have a thick peptidoglycan layer that protects them from osmotic damage. Lipopolysaccharide (**choice A**) is only found on gram-negative organisms and is associated with shock. Phospholipids (**choice C**) are found in the membranes of gram-positive and gram-negative organisms. Polysaccharide (**choice D**) can be found on gram-positive and gram-negative organisms. Teichoic acid (**choice E**) is only found on gram-positives and is used for attachment.

37. **Answer: E.** *Escherichia coli* is the most common cause of cystitis overall and should be assumed to be the cause of any case of cystitis unless contrary culture characteristics are described. It generally reduces nitrates and is also a lactose fermenter. **Choice A** identifies *Neisseria gonorrhoeae*; **choice B**, *Staphylococcus saprophyticus*; **choice C**, *Streptococcus viridans*; **choice D**, *Clostridium*.

38. **Answer: D.** This case history describes botulism (key words: home-canned green beans and visual problems). Foods classically associated are those with a neutral or alkaline pH. *C. botulinum*, the agent of botulism, is an anaerobe and thus has a low oxidation-reduction requirement. **Choice A**, *Staph aureus*; **choice B**, *Bacillus cereus*; **choice C**, *S. viridans*; **choice D**, *C. botulinum*; **choice E**, *E. coli*.

39. **Answer: A.** The disease is most likely *Mycoplasma* pneumonia caused by *Mycoplasma pneumoniae*, which is non-Gram staining and requires cholesterol for growth. **Choice B**, *C. diphtheriae*; **choice C**, *Streptococcus pyogenes*; **choice D**, *Staph aureus*; **choice E**, *Clostridium*.

40. **Answer: C**. The causal agent is *Listeria monocytogenes*. The clues are neonatal meningitis (age), gram-positive rods. The only organism in the list that is a gram-positive rod is *Listeria*. *Escherichia coli* (**choice A**) is a gram-negative rod. *Haemophilus influenzae* (**choice B**) is a gram-negative coccobacillus. *Neisseria meningitidis* (**choice D**) is a gram-negative diplococcus. *Streptococcus agalactiae* (**choice E**) is a gram-positive coccus.

41. **Answer: A**. The agent is viridans *Streptococcus*. The clues are heart valve replacement, gram-positive cocci, alpha hemolytic, and optochin resistant. *Pseudomonas aeruginosa* and *Serratia marcescens* **(choices B and C)** are gram-negative rods. *Staphylococcus aureus* **(choice D)** is a catalase-positive, gram-positive coccus. *Streptococcus pneumoniae* **(choice E)** is a gram-positive, catalase-negative coccus, but it is optochin-sensitive.

42. **Answer: A**. The clues are abscess, polymicrobial, gram-negative anaerobe. *Bacteroides* is the only anaerobe listed.

43. **Answer: D**. The causal agent is *Mycobacterium tuberculosis*. The clues are coughing up blood, acid-fast bacilli, and homeless. Sulfatides are sulfolipids which hydrolyze to form sulfuric acid. The acidic pH of the *M. tuberculosis*-containing phagosome acts to stop lysosomal fusion. Cord factor (**choice A**) is responsible for serpentine growth in vitro. Calcium dipicolinate (**choice B**) is a component of endospores. Peptidoglycan (**choice C**) is a cell wall component. Tuberculin (**choice E**) is a surface protein, which is not involved in protection from phagosome-lysosome fusion.

44. **Answer: A.** The causal agent is *Gardnerella vaginalis*. The clues are malodorous discharge, positive whiff test, thin gray discharge. Gram-negative diplococci in PMNs (**choice B**) is consistent with *Neisseria gonorrhoeae*. Koilocytic cells (**choice C**) is consistent with human papilloma virus. Owl-eye inclusions (**choice D**) are consistent with cytomegalovirus. Tzanck smears (**choice E**) are diagnostic for herpes simplex virus.

45. **Answer: E**. The causal agent is *Bacillus anthracis*. The clues are postal workers, hemoptysis and mediastinal widening. Elementary body and reticulate body (**choices A and D**) are consistent with *Chlamydia*. Endotoxin and periplasmic space (**choices B and C**) are consistent with gram-negative bacteria.

46. **Answer: C**. The clues are fist fight wound, gram-negative rods with bleach-like odor.

47. **Answer: E.** The causal agent is *Proteus vulgaris*. The clues are lower back pain (kidney stones), gram-negative rods, lactose nonfermenter, UTI. Capsules (**choice A**) are antiphagocytic, and *Proteus* does not have a capsule. Catalase **(choice B)** is produced by *Proteus*, but is not a major mechanism of pathogenesis. Coagulase (**choice C**) is produced by *Staphylococcus aureus.* Exotoxins (**choice D**) are secreted toxins.

48. **Answer: A.** The causal agent is *Clostridium difficile*. The clues are clindamycin, loose, mucoid, stools, yellow plaques. Clindamycin and other broad-spectrum antibiotics are associated with pseudomembranous colitis, as they kill off the normal gut flora and *C. difficile* flourishes without competition.

49. **Answer: A.** The patient has inclusion conjunctivitis caused by *Chlamydia trachomatis.* The only form of this bacterium that has the ability to bind to the membranes and infect is the elementary body.

50. **Answer: A**. The causal agent is *Klebsiella pneumoniae*. The clues are gelatinous and bloody sputum, PMNs, and gram-negative rods identified in the sputum. The most likely patient to present with *K. pneumoniae* would be elderly with a preexisting condition, like chronic obstructive pulmonary disease, or an alcoholic.

51. **Answer: D.** Group B *Streptococcus* (GBS) is the most common cause of neonatal meningitis, followed by *E. coli*. *S. pneumoniae* is most common in adults.

52. **Answer: A.** The clues are elderly, catheter, gram-positive cocci, catalase-negative, growth in 6.5% sodium chloride. *Staphylococcus aureus* and *Staphylococcus epidermidis* (**choices B and C**) are catalase-positive. *Streptococcus pyogenes* and viridans streptococci (**choices D and E**) would not grow in a high concentration of salt.

53. **Answer: D.** The clues are immunosuppressed (HIV+), necrotic lesion with black center and erythematous margin (ecthyma gangrenosum). *Bacillus anthracis* (**choice A**) is close because a black eschar can resemble ecthyma gangrenosum, but it usually would appear at the point of contact (probably not on the buttock). *Clostridium perfringens, Enterococcus faecalis,* and *Staphylococcus aureus* (**choices B, C,** and **E**) do not fit the case description.

54. **Answer: B.** The causal agent is *Streptococcus pyogenes*; the disease is scarlet fever. The clues are sore throat for 1 week, rash, red tongue (strawberry tongue). Gram-positive coccus, alpha hemolytic, catalase-negative (**choice A**) is descriptive of *Streptococcus pneumoniae*. Gram-positive coccus, alpha hemolytic, catalase-positive (**choice C**) does not describe an important medical pathogen. Gram-positive coccus, beta hemolytic, catalase positive (**choice D**) is descriptive of *Staphylococcus aureus*. Gram-positive coccus, gamma hemolytic, catalase negative (**choice E**) is descriptive of some strains of *Enterococcus*.

55. **Answer: D.** The causal agent is *Helicobacter pylori*. The clues are ulcer, urease positive, gram-negative curved rod. It is important to know all organisms that are associated with an increased risk of developing cancer.

56. **Answer: D.** The most likely causal agent here is a bacterium. Viral meningitis is usually mild and would not fit the CSF values. Both the age of the patient and the petechial rash suggest it is most likely to be *Neisseria meningitidis*, which is a Gram-negative diplococcus that is oxidase-positive. The overproduction of outer-membrane fragments is what leads to the petechial rash, even prior to antibiotic treatment.

57. **Answer: E.** Transposition or transpositional recombination is a form of site-specific recombination and is largely responsible for the creation of multiple drug resistant plasmids. Chromosomal drug resistance may arise by movement of a plasmid gene to the chromosome, but it is usually just a solitary gene and not a repetitive event. The Hfr chromosome arises through a single site-specific integration of a fertility factor with the bacterial chromosome.

58. **Answer: D.** This question is asking what carries the genetic code for diphtheria toxin, which must be some kind of DNA, which in turn means that the protein eEF-2 can be immediately eliminated. The diphthamide on eEF-2 is actually the substrate for the ADP-ribosylation done by the diphtheria toxin. Genes expressing the diphtheria toxin originally enter *C. diphtheriae* as part of the DNA of the temperate corynephage. Integration of this temperate phage results in a stable prophage, which directs the production of the diphtheria toxin.

59. **Answer: C.** Site-specific recombination of phage DNA into bacterial cell DNA by the process of lysogeny creates a prophage. The *recA* gene product (**choice A**) is necessary for homologous recombination with an exogenote but does not create a prophage. A virulent bacteriophage (**choice B**) causes lysis of the host cell and not the production of prophage. The lambda phage, (**choice D**), is a temperate phage, which can cause lysogeny of infected cells, but the lambda repressor is necessary in such cases to prevent the lytic life cycle. **Choice E** might be the pathway a prophage could choose to reinitiate its lytic lifestyle, but it would not be a means to create a prophage.

60. **Answer: E.** This hypothetical condition describes the mixing of one Hfr cell with 100 F^- recipients. Over time, with no cell division occurring, the one Hfr cell would repeatedly conjugate with the F^- cells and transfer one strand of its chromosomal DNA in sequence, beginning with oriT and theoretically ending with the tra genes. The most frequently transferred

bacterial genes also have the greatest likelihood of successful recombination; they are those closest to oriT; in this example, the A allele. The entire chromosome is so large that it is virtually never transferred in its entirety and thus, the tra genes would not be transferred. (Even if tra genes were transferred, oriT and tra genes have no homologous regions in the recipient cell chromosome and so would not successfully recombine within.) Thus, the recipient cell acquires only new chromosomal alleles and NOT the whole fertility factor and never changes phenotype to become an Hfr cell. Therefore, any of the answers with cell one (the Hfr parent) as the dominant type would be wrong.

The genes are transferred in linear order, so allele A will always be transferred more frequently than any of the later genes.

Therefore, given sufficient time for conjugation, the cell type that would be most numerous is that of the recipient genotype with a newly acquired allele close to oriT. This means that the best answer is choice F: cell two with a new A gene. The farther from oriT that the allele is, the less likely that it will be successfully transferred. The distractor, choice C, with all 4 alleles transferred in, is less likely.

61. **Answer: A.** The F^+ cell would contain both the bacterial chromosome and the fertility factor. The other two would just each have the bacterial chromosome (F^-) or the single DNA molecule of the chromosome with the integrated fertility factor.

62. **Answer: C.** Only F^+ and Hfr can donate genes to a recipient or F^- cell. The F^+ cell would transfer only plasmid genes. The Hfr would be the only one likely to transfer chromosomal genes.

63. **Answer: F.** In transformation, free DNA from lysed cells is not protected from the environment either by a cell or by a phage coat, but is instead naked and therefore subject to nucleases.

64. **Answer: C.** The DNA is transferred in as a linear piece and must be stabilized by homologous recombination.

65. **Answer: F.** The statement fits the definition of competency required for transformation.

66. **Answer: B.** This is generalized transduction, but what are your clues? First, it says "one phage" rather than all the phage in the cell (as for specialized). Then it also says plasmid DNA could be picked up. For specialized transduction, only episomal plasmid DNA (incorporated into the bacterial chromosome near an attachment site) or chromosomal DNA could be picked up. Finally, it mentions a lytic virus lifecycle. Lytic viruses are only capable of generalized transduction.

67. **Answer: D.** Transpositional movement actually involves a type of recombination called transposition that is a form of site-specific recombination. Site-specific recombination is also involved in integration of a temperate bacteriophage. Both transformation and transduction require homologous recombination as would transfer of Hfr DNA by conjugation. But either F factor or R factor DNA circularizes when it enters a new cell and thus is

stable without recombination since circular DNA is not subject to cellular exonucleases.

68. **Answer: A.** Choice D is a definition of lysogeny but lysogenic conversion is when lysogeny changes the characteristic of the lysogenized organism. In medicine this usually means an increased pathogenicity from the process.

69. **Answer: B.** Choice A would require phage infection with a temperate corynephage. Choice C is most likely to take place through a conjugal transfer. Choice D might occur by specialized transduction.

70. **Answer: D.** Multiple drug resistance is almost always plasmid-mediated, which rules out choices A, B, and E. Transposition is moving a piece of DNA to another molecule of DNA within the cell.

71. **Answer: C.** This is the classic description of a bacteriostatic agent.

72. **Answer: E.** The Kirby-Bauer agar disk diffusion test is a means to compare the functions of several antibiotics against one bacterial isolate. In a general sense, bacteria will be inhibited from growing in close proximity to any disk of antibiotic to which they are sensitive, so the larger the zone of inhibited growth around the filter paper disk, the more sensitive the bacteria are to that drug. Comparison between the disks cannot be accomplished without the key which comes with the kit, however, since the company which prepared the kit has done the clinical trials which correlate the in vitro results with those in human patients.

73. **Answer: E.** The MIC (Minimal Inhibitory Concentration) is the most dilute amount of drug in which no growth of a bacterial isolate will occur. The MBC (Minimal Bactericidal Concentration) of a drug is the most dilute amount of a drug in which there will be no colonial growth after the drug is removed. In some cases the MBC may be equal to the MIC, but the amount of drug necessary to kill all bacteria is never less than the amount required to inhibit their growth temporarily.

74. **Answer: E.** The disease is viral pharyngoconjunctivitis, caused by adenovirus, which is very commonly contracted through swimming pools. (Adenovirus is a naked virus and chlorination of pools does not inactivate it.) Hiking in a heavily wooded area **(choice A)** could be associated with a vector-borne disease, such as Rocky Mountain spotted fever. Eating undercooked shellfish **(choice B)** could be associated with hepatitis A or *Vibrio parahaemolyticus*, for example. Playing with toys in a day care center **(choice C)** and traveling to a developing country **(choice D)** both could begin the infection of a long list of agents.

75. **Answer: D.** The presence of hepatitis B surface antigen and the absence of the surface antibody (anti-HBs) indicate either an acute HBV infection (if patient has had the disease for only a short time) or a chronic carrier state (if the hepatitis has been going on for at least 6 months). Because acute HBV is not a choice, **choice D** then becomes the correct answer. **Choice B** would be a right answer if HBsAg had been negative and HBsAb positive. Core antibodies would not be present if the person is only vaccinated **(choice C)**. Also, HBsAg should not be present in a detectable amount

from vaccination. HBeAg and HBeAb are correlated to how contagious the patient might be, and these serologic results were not given (**choice A**).

76. **Answer: A.** The clues are lacy body rash preceded by a facial rash in a school aged child with fever. HHV-6 (**choice B**) is the causal agent of roseola, which is fever, followed by a lacy body rash in infants. Measles (**choice C**) is identified by cough, coryza, and conjunctivitis with photophobia, Koplik spots, and an exanthematous rash beginning below the ears then spreading to the trunk and extremities. Rubella (**choice D**) is the causal agent of German measles, which involves a rash beginning at the forehead and spreading down.

77. **Answer: C.** The *pol* gene codes for reverse transcriptase, integrase, and protease. Reverse transcriptase creates the provirus and integrase allows the proviral DNA to be integrated, apparently at a random site, into a chromosome in the host cell. Of the distractors, both **choices A and B** are accomplished using the host cell's RNA polymerase. **Choice D** is a function of the *gag* gene, and **choice E** is controlled by *tat* and *rev* genes.

78. **Answer: A.** The presence of HBsAg, HBcAb, and HBeAg are all indicators of an acute infection at 2 months postexposure. It is too early to identify a chronic infection (**choice B**), but the presence of HBsAg after 6 months is the main indication of a chronic infection. With a fulminant infection (**choice C**), the patient's symptoms are usually much more serious, likely a superinfection with hepatitis D or the delta agent. If he were immune (**choice D**), he would have had HBsAb in his serum. Since he has hepatitis B viral antigens in his blood, **choice E** is wrong.

79. **Answer: D.** The inmate is immune, as he has a complete complement of anti-viral antibodies.

80. **Answer: B.** This patient has herpes simplex encephalitis, which typically affects the temporal lobes. California encephalitis (**choice A**) affects older children in the middle and northwestern U.S. The polio virus (**choice C**) causes a flaccid paralysis with no sensory loss, and does not occur in the United States. St. Louis encephalitis (**choice D**) and the West Nile virus (**choice E**) usually affect older individuals.

81. **Answer: B.** The clues are transplant patient with interstitial pneumonia; CMV is the most common cause. Adenovirus (**choice A**) is associated with conjunctivitis and acute respiratory disease in military recruits, among other diseases. Although influenza (**choice C**) can cause pneumonia, there is no mention of season, and CMV is still the most common cause in transplant patients. Respiratory syncytial virus (**choice D**) is usually seen in children (especially premature infants), and rhinovirus (**choice E**) causes the common cold.

82. **Answer: A.** Polio is caused by the poliovirus. The clues are flaccid paralysis and India, and polio is transmitted by the fecal-oral route.

83. **Answer: B.** The clinical presentation is consistent with chickenpox caused by VZV. Exanthem subitum is caused by human herpes virus 6, not by CMV. Herpetic gingivostomatitis refers to herpes simplex type 1, not VZV. Infectious mononucleosis is a lymphadenopathy and herpetic whitlow is a painful herpes infection of the nail bed.

84. **Answer: E.** The only nucleic acid in the composite parental virus is the RNA belonging to poliovirus. Thus, only poliovirus is made. The only role the Coxsackie virus would play in the infection is to bind to the host cell and stimulate the uptake of the composite virus. Once uncoating takes place, the Coxsackie components play no further role. A perfect poliovirus will have been made. The progeny will have the host-cell range of polio (**choice A**) because that is what they'll be. There is no genetic material coding for the Coxsackie components, so you cannot get a composite (**choice B**). No capsid components of Coxsackie will be made; there can be no mixing (**choice C**). There was only one type of RNA; there can never be recombination (**choice D**). Sabin is a polio-specific vaccine, and poliovirus will be produced.

85. **Answer: E.** A common cause of gastroenteritis on cruise ships is the Norovirus. Norovirus is a member of the Caliciviridae family and is a nonenveloped RNA virus. Acid-fast oocysts (**choice A**) refer to persistent diarrhea caused by *Cryptosporidium parvum* or *Isospora belli*, usually seen in AIDS patients. Enveloped DNA and RNA viruses (**choices B and C**) cannot be transmitted via the fecal-oral route or live in the gastrointestinal tract because of the instability of the envelope. A nonenveloped DNA virus (**choice D**) would be consistent with adenoviral gastroenteritis, which is not the most likely cause of cruise ship gastroenteritis.

86. **Answer: B.** Gp120 is the surface antigen of HIV that mediates its attachment to CD4 lymphocytes. Gp41 is a transmembrane glycoprotein, and p24, p17, and nucleocapsid protein are all internal molecules, which would rarely be accessible to the immune response.

87. **Answer: E.** This patient has rabies, which exhibits these neurologic symptoms. In the eastern United States, the primary reservoir is raccoons.

88. **Answer: E.** An infant with difficulty breathing, wheezing, and giant multinucleated cells (syncytia) is likely to have respiratory syncytial virus. B19 (**choice A**) and influenza (**choice B**) would not show giant multinucleated cells, and B19 does not usually cause breathing difficulty. Parainfluenza (**choice C**) causes croup, which exhibits the swelling of the larynx and the seal-like barking cough. There is no mention of rash or Koplik spots, which would indicate the measles (**choice D**).

89. **Answer: E.** The virus is HSV 2, a herpesvirus, which is a dsDNA virus that uses the mechanisms of our own cells to transcribe an RNA strand from its genomic DNA and use the transcribed RNA as a messenger RNA. Of the distractors: **choice A** is the technique used by the negative-sense RNA viruses; **choice B** is used by the positive-sense RNA viruses; **choice C** is used by the retroviruses; **choice D** is used during the genomic duplication of positive sense RNA viruses; **choice F** would not produce RNA; and **choice G** is used in genomic replication by most DNA viruses.

90. **Answer: C.** The disease is mumps. The complication often seen in adult males is orchitis, which can lead to sterility.

91. **Answer: A.** CMV is an extremely common virus and crosses the placenta oftentimes without causing obvious symptoms. Fortunately, rubella, which is highly teratogenic particularly in early pregnancy, is generally prevented

by routine vaccination in childhood or at least 16 weeks prior to pregnancy. A small percentage of hepatitis B infections may occur in utero. HSV 2 will only cross the placenta if the mother acquires herpes for the first time during her pregnancy. RSV and other respiratory viruses will not. Other viruses that can cross the placenta include coxsackie B, HIV, and B19.

92. **Answer: D.** Cytomegalovirus, hepadnavirus, and poxvirus are all dsDNA viruses and not RNA. Retrovirus is an RNA virus but replicates through a dsDNA, so it also is not the correct answer. Toga is a positive RNA virus that replicates through a negative RNA intermediate and has no DNA; therefore, it's the correct answer.

93. **Answer: D.** The monospot is the most commonly used test for the diagnosis of infectious mononucleosis caused by EBV. However, it does not detect EBV-specific antibody. It instead detects heterophile antibody, which is nonspecific in that it may be present in different organisms and individuals and it cross-reacts with many animal RBCs. Epstein-Barr nuclear antigen test is not routinely run in the diagnosis of mononucleosis. Atypical lymphocytes are found in mononucleosis caused by EBV and CMV, but CMV is heterophile antibody–negative. Isolation of EBV is cumbersome and laborious, and would not distinguish previous infections from current active ones.

94. **Answer: D.** The segmented influenza viruses may undergo recombination with a similar animal virus. This leads to drastic genetic change and pandemics result from the fact that there is no underlying "herd immunity" to the new viral entity.

95. **Answer: C.** HIV. It is this genetic drift that makes it difficult for the body to fight off HIV and has complicated the development of an effective vaccine. Genetic drift is due to minor mutational change, and is possible with any organism but best described in HIV.

96. **Answer: D.** Both the symptomology, length of infection, and the epidemiological clues (college student, age 19, has given it to his girlfriend) strongly suggest that this is EBV, which is a herpesvirus.

    Choice A = parvo; **choice B** = adeno, papilloma, polyoma; **choice C** = pox; **choice D** = herpes/hepadna because there's no distinction as to circular or partial dsDNA; **choice E** = reovirus; **choice F** = arena, bunya, and orthomyxo; **choice G** = rabies; **choice H** = none; **choice I** = calici, hepe, or picorna; **choice J** = flavi and toga; **choice K** = retro.

97. **Answer: F.** The description fits congenital rubella, a togavirus, which is an enveloped positive-sense RNA virus that is not segmented.

98. **Answer: D.** If you have any trouble, think about which of these viruses has respiratory symptoms (in this case, pneumonia).

99. **Answer: C.** Remember that type 4 strains cause common warts, and these are largely transmitted by fomites or direct contact.

100. **Answer: C.** This describes the typical presentation of molluscum contagiosum, which is commonly acquired through small breaks in the skin in environments where moisture keeps the virus viable (swimming pools, showers).

101. **Answer: B.** CMV is the most common viral cause of death in bone-marrow transplant patients, causing an interstitial pneumonia.

102. **Answer: D.** Perinatal infection with human papilloma virus can cause infantile laryngeal warts.

103. **Answer: B.** Cutaneous candidiasis is a problem in skin folds of obese individuals. It is an even greater problem in diabetic patients because of the high sugar levels. Only the members of the genus *Candida* would produce a creamy surface growth. The erythematous base is due to the production of a cytotoxin. *Aspergillus, Epidermophyton,* and *Microsporum* are all monomorphic filamentous fungi and would not fit the description. *Sporothrix* is found as cigar-shaped budding yeasts but would not clinically present like this. It is traumatically implanted to start subcutaneous infections.

104. **Answer: E.** The causal agent is *Coccidioides immitis*. The clues are southwest U.S., migrant worker (works outside), erythematous skin rash (erythema nodosum). The diagnostic form of *C. immitis* in the sputum is a spherule. Broad-based budding yeast (**choice A**) describes *Blastomyces dermatitidis*. Monomorphic encapsulated yeast (**choice B**) describes *Cryptococcus neoformans*. Nonseptate hyphae with broad angles (**choice C**) describes *Mucor* species. Septate hyphae dichotomously branching at acute angles (**choice D**) describes *Aspergillus fumigatus*.

105. **Answer: C.** The clues here are the geography, weight loss, granulomatous inflammation, and macrophages engorged with oval structures (RES disease). The colonial appearance and tuberculate spores strongly suggest the causal agent to be *Histoplasma capsulatum. Histoplasma* is acquired from dusty environments containing bird (most often chicken or starling) or bat feces. The areas of highest endemicity are in the great central river beds with bat caves, chicken coops, and starling roosts having extremely high levels.

106. **Answer: D.** The causal agent is *Blastomyces dermatitidis*. The clues are southeastern U.S. and a sputum smear revealing thick-walled, refractile, double-contoured yeast cells (another way to say broad-based budding). Remember that the name of the organism contains the site of dissemination.

107. **Answer: A.** The clues are immunocompromised, myocardial infarction, and septate acutely branching hyphae in nearly every tissue. Remember, *Aspergillus* is extremely invasive in immunocompromised individuals.

108. **Answer: D.** The causal agent is *Cryptococcus neoformans*. The clues are HIV, headache and blurred vision, encapsulated organisms in CSF. *C. neoformans* is the number one cause of meningitis in AIDS patients. You should know that it is monomorphic. “Spaghetti and meatballs” KOH (**choice A**) describes *Malassezia furfur*. Branching septate hyphae (**choice B**) describes *Aspergillus*. Mycelial form at room temperature and a yeast at 37°C (**choice C**) describes all of the dimorphic fungi. Nonencapsulated dimorphic yeast that reproduces by budding (**choice E**) describes *Blastomyces*.

109. **Answer: D.** The clues are AIDS patient and atypical pneumonia. *Pneumocystis jirovecii* is the hallmark atypical pneumonia of AIDS.

110. **Answer: C.** This patient is suffering from *Plasmodium falciparum* malaria acquired shortly before her departure from Kenya. Liver stages of *Plasmodium* are not susceptible to chloroquine killing. Because she did not continue the prophylaxis after her return to the States, those parasites were allowed to initiate all of the erythrocytic stages of the life cycle. Any erythrocytic stages generated out of the liver phase of the life cycle while she remained on prophylaxis would have been killed. Thus, the late onset of her symptoms was due to survival of exoerythrocytic stages that had not yet left the liver at the time she ceased prophylaxis. Hypnozoites (**choice A**) are responsible for relapse of symptoms in *P. vivax* and *P. ovale* malarias but do not exist in *P. falciparum*, and it is clear that she has *falciparum* malaria due to the delicate ring forms multiply infecting erythrocytes and the sausage-shaped gametocytes. Sporozoites (**choice B**) are the infectious forms injected by mosquitoes and would not have been available in this country to initiate the symptoms on the time course described. Erythrocytic schizonts and merozoites (**choices D and E**) would have been killed by prophylaxis before she left Africa and could not be responsible for the late onset of symptoms.

111. **Answer: D.** This child has the typical symptoms of hookworm disease, caused in this country usually by *Necator americanus*. The infection is acquired by penetration of the filariform larvae through the skin of the feet or buttocks, after contamination of soil with the eggs of the agent deposited in human feces. Of the other distractors, **choice A** would be most likely if the infection were due to ascarids, pinworms, or whipworms. **Choice C** would describe infection with either *Taenia* or *Trichinella*, and **choice E** would be the means of infection with *Plasmodium*.

112. **Answer: E.** The described infection could be *Cryptosporidium*, *Isospora*, *Microsporidia*, or *Cyclospora*, which are very difficult infections in AIDS patients even though they are self-resolving in normal noncompromised individuals. In AIDS patients they are most commonly unrelenting, even with treatment. They are usually acquired from water. *Toxoplasma* (**choice D**) is from cats.

113. **Answer: D.** The positive IgM titer arising in the eighth month means that this woman has become acutely infected with *Toxoplasma*. Infections acquired at this time have a high likelihood of infecting the fetus and are most likely to be manifested by the development of retinochoroiditis. A mother can transmit this parasite to her fetus only during an acute infection; therefore, all future fetuses will be protected from the disease (**choice A**). Because IgG antibodies cross the placenta (**choice B**), presence of the anti-*Toxoplasma* antibodies of this class in the neonate may simply reflect the infection of the mother—only a positive IgM response in the neonate is proof of the child's infection, which should therefore be treated. There is no way to reverse major organ damage (**choice E**) when it occurs in utero, but it would not be expected to occur with an acute infection beginning in the third trimester.

114. **Answer: F.** *Leishmania* spp. are transmitted by the bite of sandflies. They cannot be transmitted from person to person by trivial means, so unless organ transplantation is occurring in the barracks, direct human contact (**choice E**) is not a possibility. To survive outside the human host, they must be in the vector (sandfly), so transmission by food or water (**choices A** or **D**) is not possible. Of the distractors that involve true vectors: *Anopheles*

mosquitoes (**choice B**) transmit malaria; reduviid bugs (**choice C**) transfer American trypanosomiasis (Chagas disease); and tsetse flies (**choice G**) transmit African trypanosomiasis (sleeping sickness).

115. **Answer: C.** *Giardia* is common in mountain streams throughout the U.S., and the presentation of prolonged fatty diarrhea and weight loss is pathognomonic. It causes its pathology by its adherence to the mucosa of the upper small intestine with its ventral sucking disc. No toxic metabolites or lytic enzymes are involved in the pathology, which apparently results from blockage of normal fat digestion. The organism is a flagellate, and thus has flagella, but migration into extraintestinal sites is not a well known problem associated with pathology. And although the organism does encyst as it passes along the intestine, this is not known to produce symptoms.

116. **Answer: B.** The clues are bloody diarrhea, fever, bacterial cultures negative, organisms with RBCs inside them. *Cryptosporidium parvum* (**choice A**) is typically found in AIDS patients. *Giardia lamblia* (**choice C**) is associated with fatty, foul smelling diarrhea. *Toxoplasma gondii* (**choice D**) would likely cause a flu-like illness in this age group if acquired as primary infection; if acquired in utero, might cause blindness later in life. You can rule out *Shigella dysenteriae* (**choice E**), because bacterial cultures were negative.

117. **Answer: C.** The clues are Egypt, blood in urine, eggs with terminal spines, working in rice field (indicates his possible exposure to contaminated water). Also, be aware that in Africa, *S. haematobium* is associated with bladder cancer. *Entamoeba histolytica* (**choice A**) would cause bloody stool, not urine. *Fasciolopsis buski* (**choice B**) is the intestinal fluke; eggs do not have terminal spines. *S. japonicum* and *S. mansoni* (**choices D** and **E**) are intestinal schistosomes and would not cause blood in urine; the egg for *S. mansoni* has a subterminal spine, whereas the egg for *S. japonicum* is fat and oval with one tiny lateral spine.

118. **Answer: D.** The clues are frothy, yellow discharge, itching, organisms identified on wet mount, bacterial cultures were negative. With *Candida albicans* (**choice A**), the discharge would have been white and creamy. *Trichophyton* (**choice B**) causes skin, hair, and nail infections and is a cutaneous fungus. *Chlamydia trachomatis* (**choice C**) would not be visible on wet mount and causes intracellular infection of epithelial cells. *Giardia lamblia* (**choice E**) is associated with diarrhea.

119. **Answer: B.** The clues are missionary, high fever, chills, Africa, multiple ring structures, and crescent-shaped gametes. *Leishmania* **(choice A)** produces amastigotes inside phagocytic cells and causes either visceral, cutaneous, or mucocutaneous pathology. *Plasmodium malariae* (**choice C**) clues might include bar and band forms in RBCs, and 72 hour fever spikes. In *P. ovale* and *P. vivax* (**choices D** and **E**) there will be Schüffner dots in RBCs.

120. **Answer: D**. The clues are perianal itching, irritable during the day, not sleeping at night, eggs with flattened side, and Scotch tape test.

121. **Answer: E.** The clues are tropical country, prolapsed rectum, worms resembling whips, barrel shaped eggs with bipolar plugs. The common name for *Trichuris* is whipworm.

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Fundamentals of Pathology 1

Learning Objectives

- ❏ Define the etiology, pathogenesis, morphology, and clinical significance of disease
- ❏ List techniques for staining pathologic specimens

OVERVIEW OF PATHOLOGY

Definitions

- The study of the essential nature of disease, including symptoms/signs, pathogenesis, complications, and morphologic consequences such as structural and functional alterations in cells, tissues, and organs
- The study of all aspects of the disease process focusing on the pathogenesis leading to classical structural changes (gross and histopathology) and molecular alterations

The **etiology** (cause) of a disease may be genetic or environmental. The **pathogenesis** of a disease defines the temporal sequence and the patterns of cellular injury that lead to disease. **Morphologic** changes of the disease process include both gross changes and microscopic changes. The **clinical significance** of a disease relates to its signs and symptoms, disease course including complications, and prognosis.

Methods Used

Gross examination of organs on exam questions has 2 major components: identifying the organ and identifying the pathology. Useful gross features include consideration of size, shape, consistency, and color.

Microscopic examination of tissue

- In light microscopic examination of tissue, **hematoxylin and eosin (H&E)** is considered the gold standard stain and is used routinely in the initial microscopic examination of pathologic specimens.

  The common denominator of the features is that hematoxylin binds nucleic acids and calcium salts, while eosin stains the majority of proteins (both extracellular and intracellular).

Table 1-1. Structures Stained by Hematoxylin and Eosin

Hematoxylin	Eosin
Stains blue to purple	Stains pink to red
• Nuclei	• Cytoplasm
• Nucleoli	• Collagen
• Bacteria	• Fibrin
• Calcium	• RBCs
	• Thyroid colloid

- **Other histochemical stains** (chemical reactions): Prussian blue (stains iron), Congo red (stains amyloid), acid fast (Ziehl-Neelsen, Fite) (stains acid-fast bacilli), periodic acid-Schiff (PAS, stains high carbohydrate content molecules), Gram stain (stains bacteria), trichrome (stains cells and connective tissue), and reticulin (stains collagen type III molecules).

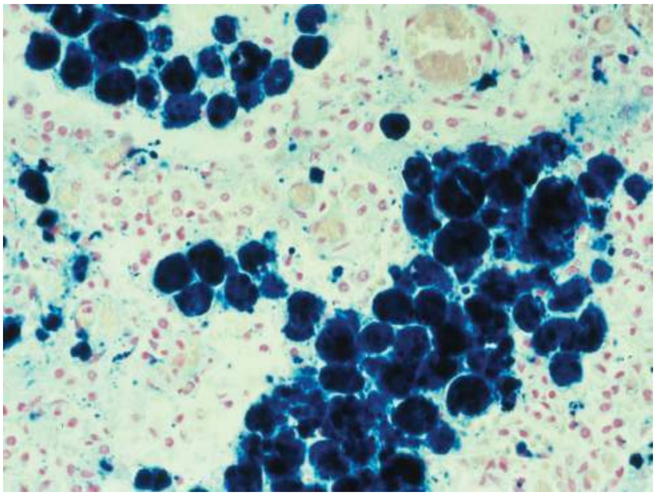

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Figure 1-1. Prussian Blue Stain Showing Hemosiderin, Which Results from RBC Breakdown Within Macrophages

- **Immunohistochemical (antibody) stains** include cytokeratin (stains epithelial cells), vimentin (stains cells of mesenchymal origin except the 3 muscle types; stains many sarcomas), desmin (stains smooth, cardiac, and skeletal myosin), prostate specific antigen, and many others.

Ancillary techniques include **immunofluorescence microscopy (IFM)**, typically used for renal and autoimmune disease, and **transmission electron microscopy (EM)**, used for renal disease, neoplasms, infections, and genetic disorders.

Molecular techniques include protein electrophoresis, Southern and Western blots, polymerase chain reaction (PCR), and cytogenetic analysis (karyotyping, in situ hybridization studies).

Cellular Injury and Adaptation 2

Learning Objectives

- ❑ Explain causes of cellular injury
- ❑ Demonstrate understanding of cellular changes during injury and cell death
- ❑ Answer questions about cellular adaptive responses to injury
- ❑ Describe cellular alterations during injury

CAUSES OF CELLULAR INJURY

Hypoxia is the most common cause of injury; it occurs when lack of oxygen prevents the cell from synthesizing sufficient ATP by aerobic oxidation. Major mechanisms leading to hypoxia are ischemia, cardiopulmonary failure, and decreased oxygen-carrying capacity of the blood (e.g., anemia). **Ischemia**, due to a loss of blood supply, is the most common cause of hypoxia and is typically related to decreased arterial flow or decreased venous outflow (e.g., atherosclerosis, thrombus, thromboembolus).

Pathogens (viruses, bacteria, parasites, fungi, and prions) can injure the body by direct infection of cells, production of toxins, or host inflammatory response.

Immunologic dysfunction includes hypersensitivity reactions and autoimmune diseases.

Congenital disorders are inherited genetic mutations (e.g., inborn errors of metabolism).

Chemical injury can occur with drugs, poisons (cyanide, arsenic, mercury, etc.), pollution, occupational exposure (CCl_4, asbestos, carbon monoxide, etc.), and social/lifestyle choices (alcohol, smoking, IV drug abuse, etc.)

Physical forms of injury include trauma (blunt/penetrating/crush injuries, gunshot wounds, etc.), burns, frostbite, radiation, and pressure changes.

Nutritional or vitamin imbalance

- **Inadequate calorie/protein intake** can cause marasmus (decrease in total caloric intake), and kwashiorkor (decrease in total protein intake).
- **Excess caloric intake** can cause obesity (second leading cause of premature preventable death in the United States) and atherosclerosis.
- **Vitamin deficiencies** can be seen with vitamin A (night blindness, squamous metaplasia, immune deficiency), vitamin C (scurvy), vitamin D (rickets and osteomalacia), vitamin K (bleeding diathesis), vitamin B12 (megaloblastic anemia, neuropathy, and spinal cord degeneration), folate (megaloblastic anemia and neural tube defects), and niacin (pellagra [diarrhea, dermatitis, and dementia]).

- **Hypervitaminosis** is less commonly a problem but can result in tissue-specific abnormalities.

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Figure 2-1. Radiograph of a Child with Rickets Shows Bowed Legs

CELLULAR CHANGES DURING INJURY

Cellular responses to injury include adaptation (hypertrophy or atrophy, hyperplasia or metaplasia), reversible injury, and irreversible injury and cell death (necrosis, apoptosis, or necroptosis).

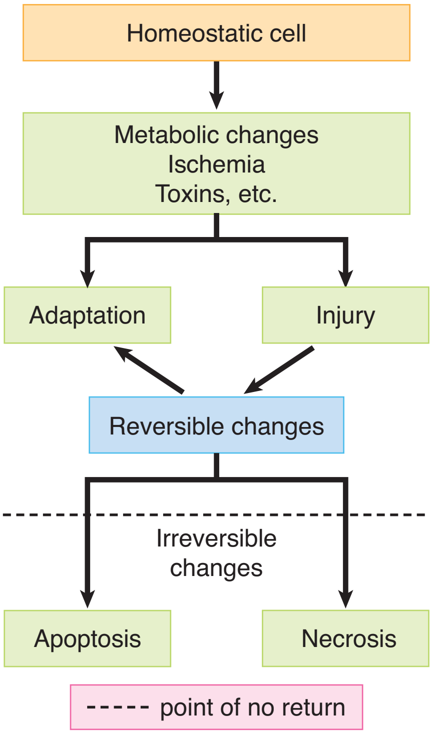

Figure 2-2. Cellular Response to Stress and Injurious Stimuli

The **cellular response to injury depends on several important factors**, including the type of injury, duration (including pattern) of injury, severity and intensity of injury, type of cell injured, the cell's metabolic state, and the cell's ability to adapt.

The **critical intracellular targets that are susceptible to injury** are DNA, production of ATP via aerobic respiration, cell membranes, and protein synthesis.

Important mechanisms of cell injury are as follows:

- Damage to DNA, proteins, lipid membranes, and circulating lipids (LDL) can be caused by oxygen-derived free radicals, including superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2).
- ATP depletion: Several key biochemical pathways are dependent on ATP. Disruption of Na^+/K^+ or Ca^{++} pumps cause imbalances in solute concentrations. Additionally, ATP depletion increases anaerobic glycolysis that leads to a decrease in cellular pH. Chronic ATP depletion causes morphological and functional changes to the ER and ribosomes.
- Increased cell membrane permeability: Several defects can lead to movement of fluids into the cell, including formation of the membrane attack complex via complement, breakdown of Na+/K+ gradients (i.e., causing sodium to enter or potassium to leave the cell), etc.
- Influx of calcium can cause problems because calcium is a second messenger, which can activate a wide spectrum of enzymes. These enzymes include proteases (protein breakdown), ATPases (contributes to ATP depletion), phospholipases (cell membrane injury), and endonucleases (DNA damage).
- Mitochondrial dysfunction causes decreased oxidative phosphorylation and ATP production, formation of mitochondrial permeability transition (MPT) channels, and release of cytochrome c (a trigger for apoptosis).

Note

Protective factors against free radicals include:

- Antioxidants
  Vitamins A, E, and C
- Superoxide dismutase
  Superoxide → hydrogen peroxide
- Glutathione peroxidase
  Hydroxyl ions or hydrogen peroxide → water
- Catalase
  Hydrogen peroxide → oxygen and water

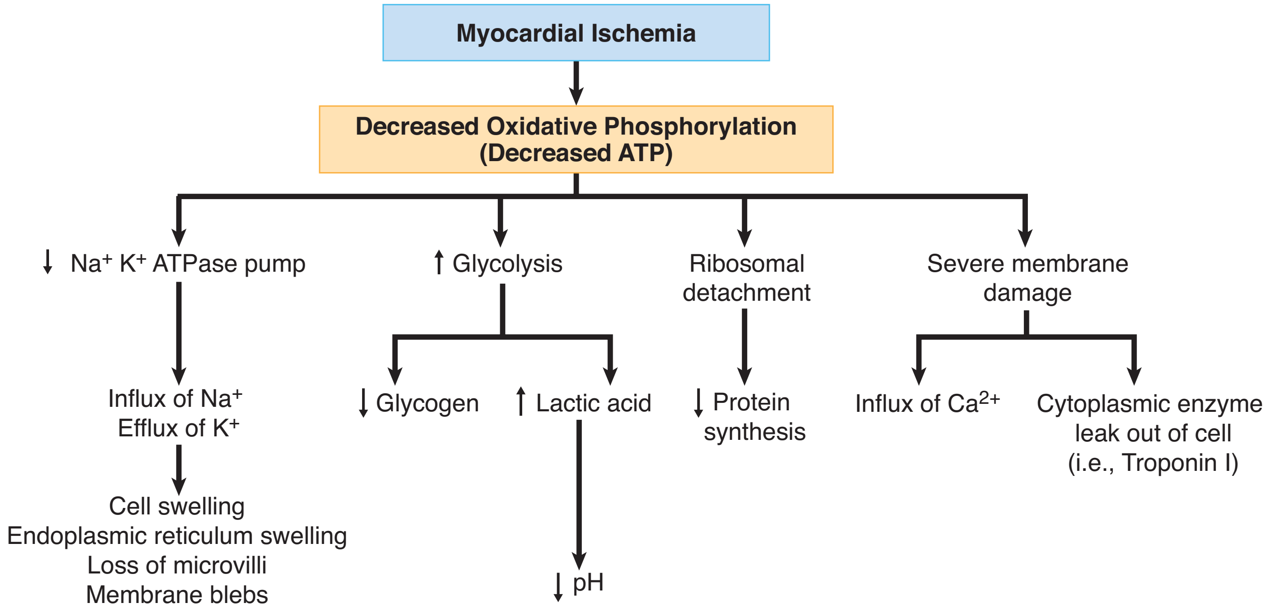

Figure 2-3. Classic Example of Cellular Injury Caused by Hypoxia

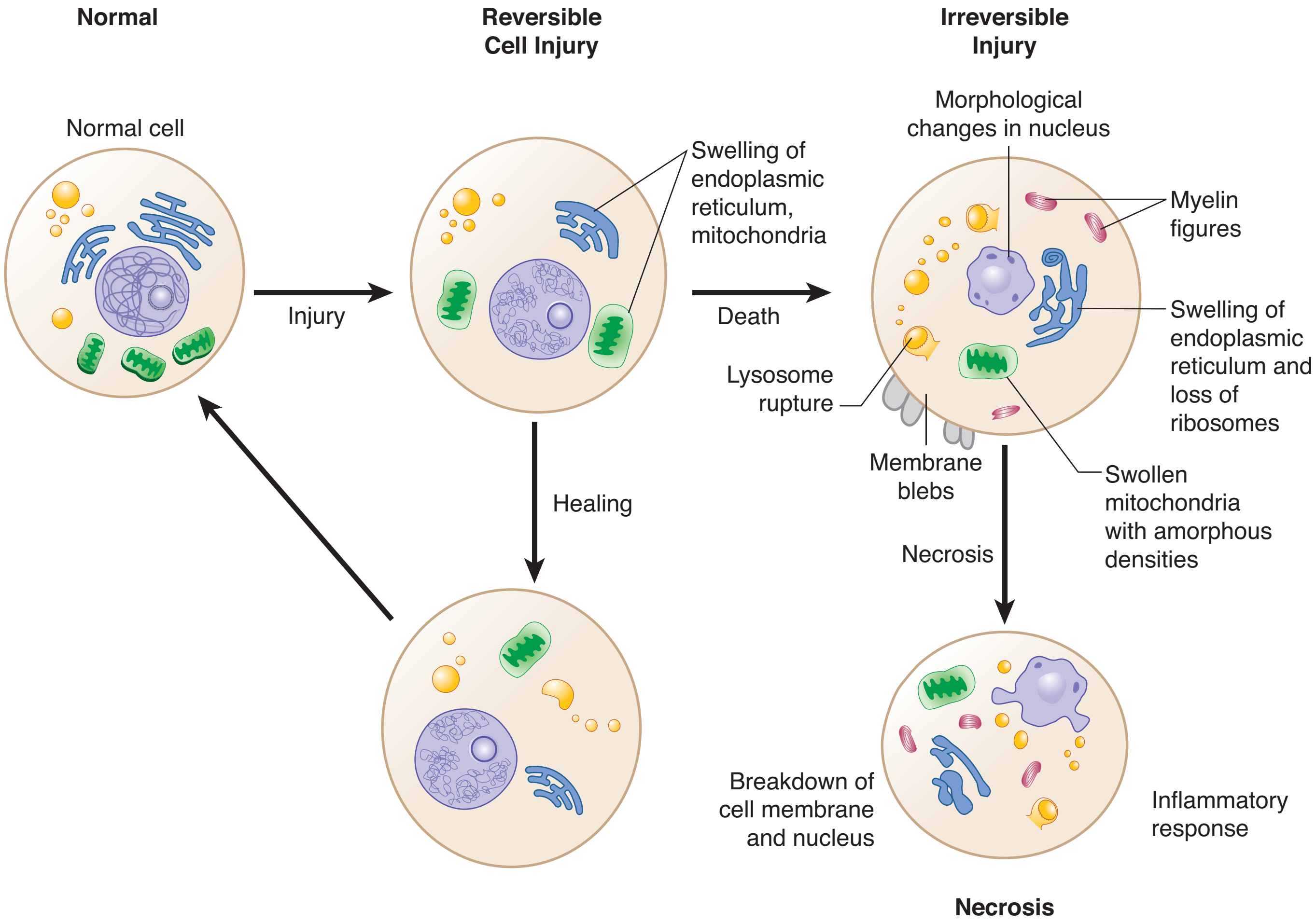

Figure 2-4. Cell Injury

Note

Reversible and irreversible changes represent a spectrum. Keep in mind that any of the reversible changes can become irreversible.

Clinical Correlate

The loss of membrane integrity (cell death) allows intracellular enzymes to leak out, which can then be measured in the blood. Detection of these proteins in the circulation serves as a clinical marker of cell death and organ injury. Clinically important examples:

- Myocardial injury: troponin (most specific), CPK-MB, lactate dehydrogenase (LDH)
- Hepatitis: transaminases
- Pancreatitis: amylase and lipase
- Biliary tract obstruction: alkaline phosphatase

Reversible cell injury:

- **Decreased synthesis of ATP** by oxidative phosphorylation.
- **Decreased function of Na+K+ ATPase membrane pumps**, which in turn causes influx of Na^+ and water, efflux of K^+, cellular swelling (hydropic swelling), and swelling of the endoplasmic reticulum.
- The **switch to anaerobic glycolysis** results in depletion of cytoplasmic glycogen, increased lactic acid production, and decreased intracellular pH.
- **Decreased protein synthesis** leads to detachment of ribosomes from the rough endoplasmic reticulum.
- **Plasma-membrane blebs and myelin figures** may be seen.

Irreversible cell injury:

- **Severe membrane damage** plays a critical role in irreversible injury, allows a massive influx of calcium into the cell, and allows efflux of intracellular enzymes and proteins into the circulation.
- **Marked mitochondrial dysfunction** produces mitochondrial swelling, large densities seen within the mitochondrial matrix, irreparable damage of the oxidative phosphorylation pathway, and an inability to produce ATP.

- **Rupture of the lysosomes** causes release of lysosomal digestive enzymes into the cytosol and activation of acid hydrolases followed by autolysis.
- **Nuclear changes** can include pyknosis (degeneration and condensation of nuclear chromatin), karyorrhexis (nuclear fragmentation), and karyolysis (dissolution of the nucleus).

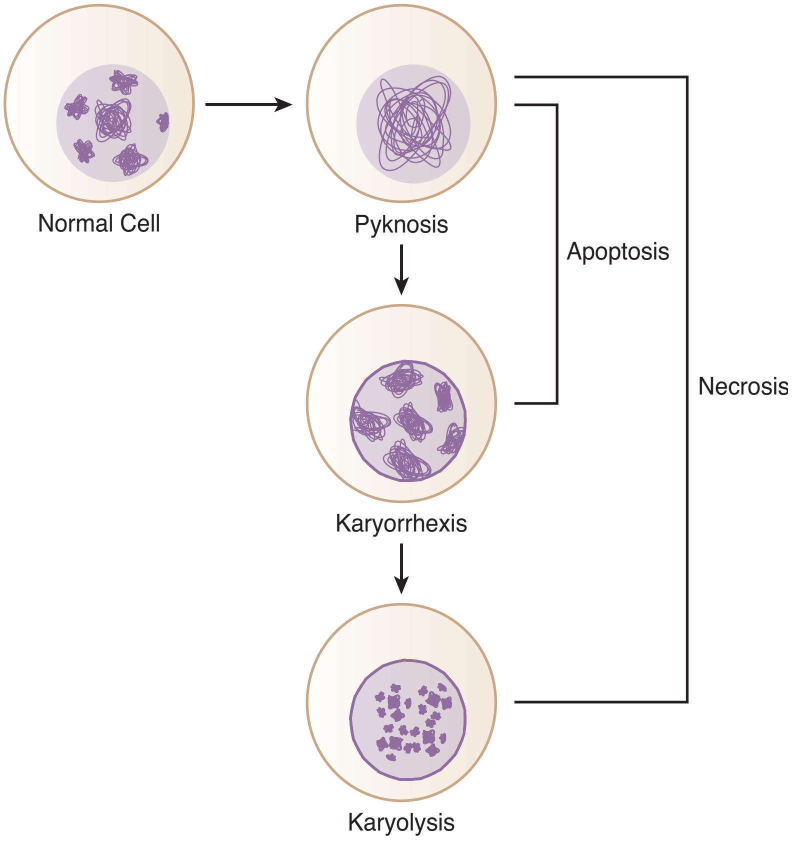

Figure 2-5. Nuclear Changes in Irreversible Cell Injury

CELL DEATH

Morphologic types of necrosis (cell death in living tissue, often with an inflammatory response) are as follows:

- **Coagulative necrosis**, the most common form of necrosis, is most often due to ischemic injury (infarct). It is caused by the denaturing of proteins within the cytoplasm. Microscopic examination shows loss of the nucleus but preservation of cellular shape. Coagulative necrosis is common in most organs, including the heart, liver, and kidney, but not the brain.
- **Liquefaction necrosis** results from cellular destruction by hydrolytic enzymes, leading to autolysis (release of proteolytic enzymes from injured cells) and heterolysis (release of proteolytic enzymes from inflammatory cells). Liquefaction necrosis occurs in abscesses, brain infarcts, and pancreatic necrosis.
- **Caseous necrosis** is a combination of coagulation and liquefaction necrosis. The gross appearance is soft, friable, and "cheese-like." Caseous necrosis is characteristic of granulomatous diseases, including tuberculosis.

Note

Liquefaction by leukocyte enzymes is called suppuration, and the resultant fluid is called pus.

Bridge to Biochemistry

Damage to fat cells releases triglycerides. The triglycerides are broken down by the action of lipases to fatty acids. The fatty acids may associate with calcium and form calcium soaps (saponification).

Note

Necrotic tissue within the body evokes an inflammatory response that removes the dead tissue and is followed by healing and tissue repair. Necrotic debris may also undergo dystrophic calcification.

- **Fat necrosis** is caused by the action of lipases on adipocytes and is characteristic of acute pancreatitis. On gross examination fat necrosis has a chalky white appearance.
- **Fibrinoid necrosis** is a form of necrotic connective tissue that histologically resembles fibrin. On microscopic examination fibrinoid necrosis has an eosinophilic (pink) homogeneous appearance. It is often due to acute immunologic injury (e.g., hypersensitivity type reactions II and III) and vascular hypertensive damage.
- **Gangrenous necrosis** is a gross term used to describe dead tissue. Common sites of involvement include lower limbs, gallbladder, GI tract, and testes. Dry gangrene has coagulative necrosis for the microscopic pattern, while wet gangrene has liquefactive necrosis.

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Figure 2-6. Gangrenous Necrosis Affects the First and Third Toes of a Diabetic Foot

Clinical Correlate

- If the cells in the interdigital space fail to undergo apoptosis, the fetus will be born with webbed hands and/or webbed feet, a condition known as *syndactyly*.
- Another example is the hormone-dependent apoptosis prior to menstruation; programmed cell death plays a role in endometrial gland morphological changes.

Apoptosis is a specialized form of programmed cell death without an inflammatory response. It is an active process regulated by proteins that often affects only single cells or small groups of cells.

- In **morphologic appearance,** the cell shrinks in size and has dense eosinophilic cytoplasm. Next, nuclear chromatin condensation (pyknosis) is seen that is followed by fragmentation of the nucleus (karyorrhexis). Cytoplasmic membrane blebs form next, leading eventually to a breakdown of the cell into fragments (apoptotic bodies). Phagocytosis of apoptotic bodies is by adjacent cells or macrophages.
- **Stimuli for apoptosis** include cell injury and DNA damage, lack of hormones, cytokines, or growth factors, and receptor-ligand signals such as Fas binding to the Fas ligand and tumor necrosis factor (TNF) binding to TNF receptor 1 (TNFR1).
- **Apoptosis is regulated by proteins.** The protein bcl-2 (which inhibits apoptosis) prevents release of cytochrome c from mitochondria and binds pro-apoptotic protease activating factor (Apaf-1). The protein p53 (which stimulates apoptosis) is elevated by DNA injury and arrests the cell cycle. If DNA repair is impossible, p53 stimulates apoptosis.

- **Execution of apoptosis** is mediated by a cascade of caspases (cysteine aspartic acid proteases). The caspases digest nuclear and cytoskeletal proteins and also activate endonucleases.
- **Physiologic examples of apoptosis** include embryogenesis (organogenesis and development), hormone-dependent apoptosis (menstrual cycle), thymus (selective death of lymphocytes).
- **Pathologic examples of apoptosis** include viral diseases (viral hepatitis [Councilman body]), graft-versus-host disease, and cystic fibrosis (duct obstruction and pancreatic atrophy).

Serum enzyme markers of cell damage include aspartate aminotransferase (AST) (liver injury), alanine aminotransferase (ALT) (liver injury), creatine kinase (CK-MB) (heart injury), and amylase and lipase (pancreatic injury; amylase also rises with salivary gland injury).

Clinical Correlate

Graft-versus-host disease (GVHD) is an example of apoptosis which occurs in allogeneic hematopoietic stem cell transplant recipients. The transplanted marrow has cytotoxic T-cells which recognize the new host proteins (usually HLA) as foreign. Organs typically involved include the skin, mucosa, liver, and GI tract. The histologic hallmark of GVHD is apoptosis.

CELLULAR ADAPTIVE RESPONSES TO INJURY

In general, cellular adaptation is a potentially reversible change in response to the environment.

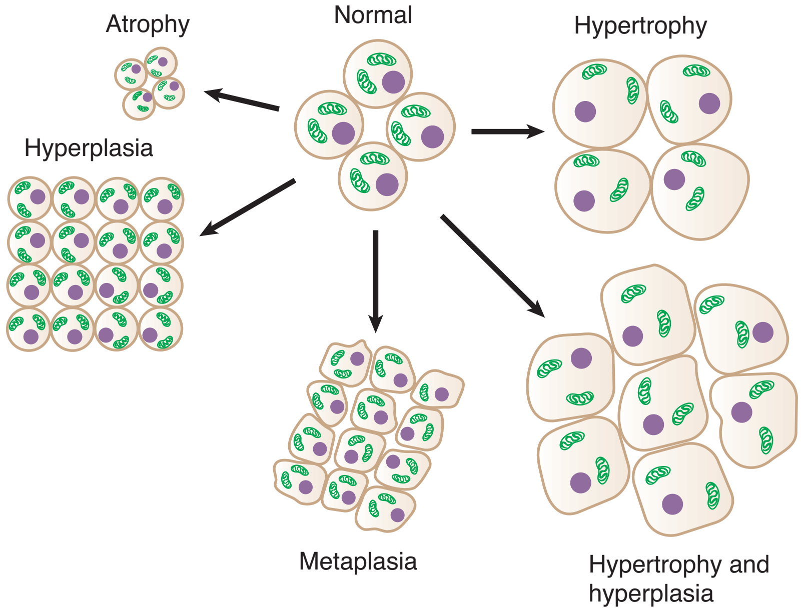


Figure 2-7. Cellular Adaptive Responses to Cell Injury

Atrophy is a decrease in cell/organ size and functional ability. Causes of atrophy include decreased workload/disuse (immobilization); ischemia (atherosclerosis); lack of hormonal or neural stimulation, malnutrition, and aging.

Light microscopic examination shows small shrunken cells with lipofuscin granules. Electron microscopy shows decreased intracellular components and autophagosomes.

Hypertrophy is an increase in cell size and functional ability due to increased synthesis of intracellular components.

Causes of hypertrophy include:

- Increased mechanical demand can be physiologic (striated muscle of weight lifters) or pathologic (cardiac muscle in hypertension).
- Increased endocrine stimulation plays a role in puberty (growth hormone, androgens/estrogens, etc.), gravid uterus (estrogen), and lactating breast (prolactin and estrogen).

Hypertrophy is mediated by growth factors, cytokines, and other trophic stimuli and leads to increased expression of genes and increased protein synthesis.

Hypertrophy and hyperplasia often occur together.

Clinical Correlate

Residence at high altitude, where oxygen content of air is relatively low, leads to compensatory hyperplasia of red blood cell precursors in the bone marrow and an increase in the number of circulating red blood cells (secondary polycythemia).

Hyperplasia is an increase in the number of cells in a tissue or organ. Some cell types are unable to exhibit hyperplasia (e.g., nerve, cardiac, skeletal muscle cells).

- Physiologic causes of hyperplasia include compensatory mechanisms (e.g., after partial hepatectomy), hormonal stimulation (e.g., breast development at puberty), and antigenic stimulation (e.g., lymphoid hyperplasia).
- Pathologic causes of hyperplasia include endometrial hyperplasia and prostatic hyperplasia of aging.

Hyperplasia is mediated by growth factors, cytokines, and other trophic stimuli; increased expression of growth-promoting genes (proto-oncogenes); and increased DNA synthesis and cell division.

Clinical Correlate

Barrett esophagus is a classic example of metaplasia. The esophageal epithelium is normally squamous, but it undergoes a change to intestinal epithelium (columnar) when it is under constant contact with gastric acid.

Metaplasia is a reversible change of one fully differentiated cell type to another, usually in response to irritation. It has been suggested that the replacement cell is better able to tolerate the environmental stresses. For example, bronchial epithelium undergoes squamous metaplasia in response to the chronic irritation of tobacco smoke.

The proposed mechanism is that the reserve cells (or stem cells) of the irritated tissue differentiate into a more protective cell type due to the influence of growth factors, cytokines, and matrix components.

OTHER CELLULAR ALTERATIONS DURING INJURY

Pathologic accumulations

- **Lipids** that can accumulate intracellularly include triglycerides (e.g., fatty change in liver cells), cholesterol (e.g., atherosclerosis, xanthomas), and complex lipids (e.g., sphingolipid accumulation).
- **Proteins** can accumulate in proximal renal tubules in proteinuria and can form Russell bodies (intracytoplasmic accumulation of immunoglobulins) in plasma cells.
- **Glycogen storage diseases** (*See* Genetic Disorders chapter.)
- **Exogenous pigments** include anthracotic pigmentation of the lung (secondary to the inhalation of carbon dust), tattoos, and lead that has been ingested (e.g., gingival lead line, renal tubular lead deposits).

Endogenous pigments

- **Lipofuscin** is a wear-and-tear pigment that is seen as perinuclear yellow-brown pigment. It is due to indigestible material within lysosomes and is common in the liver and heart.
- **Melanin** is a black-brown pigment derived from tyrosine found in melanocytes and substantia nigra.
- **Hemosiderin** is a golden yellow-brown granular pigment found in areas of hemorrhage or bruises. Systemic iron overload can lead to hemosiderosis (increase in total body iron stores without tissue injury) or hemochromatosis (increase in total body iron stores with tissue injury). Prussian blue stain can identify the iron in the hemosiderin.
- **Bilirubin** accumulates in newborns in the basal ganglia, causing permanent damage (kernicterus).

Hyaline change is a nonspecific term used to describe any intracellular or extracellular alteration that has a pink homogenous appearance (proteins) on H&E stains.

- Examples of **intracellular hyaline** include renal proximal tubule protein reabsorption droplets, Russell bodies, and alcoholic hyaline.
- Examples of **extracellular hyaline** include hyaline arteriolosclerosis, amyloid, and hyaline membrane disease of the newborn.

Pathologic forms of calcification

- **Dystrophic calcification** is the precipitation of calcium phosphate in dying or necrotic tissues. Examples include fat necrosis (saponification), psammoma bodies (laminated calcifications that occur in meningiomas and papillary carcinomas of the thyroid and ovary), Mönckeberg medial calcific sclerosis in arterial walls, and atherosclerotic plaques.
- **Metastatic calcification** is the precipitation of calcium phosphate in normal tissue due to hypercalcemia (supersaturated solution). The many causes include hyperparathyroidism, parathyroid adenomas, renal failure, paraneoplastic syndrome, vitamin D intoxication, milk-alkali syndrome, sarcoidosis, Paget disease, multiple myeloma, metastatic cancer to the bone. The calcifications are located in the interstitial tissues of the stomach, kidneys, lungs, and blood vessels.

Inflammation 3

Learning Objectives

- ❑ Solve problems concerning acute and chronic inflammation
- ❑ Describe tissue responses to infectious agents

ACUTE INFLAMMATION

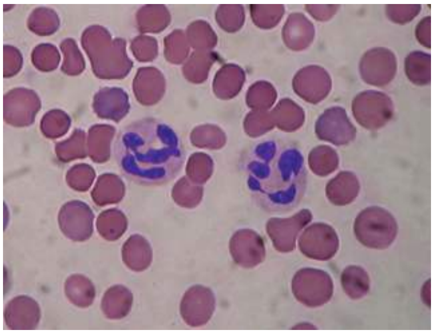
Acute inflammation is an immediate response to injury or infection, which is part of innate immunity.

- Short duration in normal host
- Cardinal signs of inflammation include rubor (redness); calor (heat); tumor (swelling); dolor (pain); functio laesa (loss of function).

The important components of acute inflammation are hemodynamic changes, **neutrophils**, and chemical mediators.

Hemodynamic Changes

- Initial transient vasoconstriction
- Massive vasodilatation mediated by histamine, bradykinin, and prostaglandins
- Increased vascular permeability
  - Chemical mediators of increased permeability include vasoactive amines (histamine and serotonin), bradykinin (an end-product of the kinin cascade), leukotrienes (e.g., LTC4, LTD4, LTE4).
  - The mechanism of increased vascular permeability involves endothelial cell and pericyte contraction; direct endothelial cell injury; and leukocyte injury of endothelium.
- Blood flow slows (stasis) due to increased viscosity, allows neutrophils to marginate



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Lobed nucleus, small granules

Neutrophil

Neutrophils

- Life span in tissue 1–2 days
- Synonyms: segmented neutrophils, polymorphonuclear leukocytes (PMN)
- Primary (azurophilic) granules contain myeloperoxidase, phospholipase A2, lysozyme (damages bacterial cell walls by catalyzing hydrolysis of 1,4-beta- linkages), and acid hydrolases. Also present are elastase, defensins (microbicidal peptides active against many gram-negative and gram-positive bacteria, fungi, and enveloped viruses), and bactericidal permeability increasing protein (BPI).
- Secondary (specific) granules contain phospholipase A2, lysozyme, leukocyte alkaline phosphatase (LAP), collagenase, lactoferrin (chelates iron), and vitamin B12-binding proteins.
- **Macrophages** (life span in tissue compartment is 60–120 days) have acid hydrolases, elastase, and collagenase.

Clinical Correlate

- A normal mature neutrophil has a segmented nucleus (3–4 segments).
- Hypersegmented neutrophils (>5 segments) are thought to be pathognomonic of the class of anemias called megaloblastic anemias (vitamin B12 or folate deficiencies).

Neutrophil margination and adhesion. Adhesion is mediated by complementary molecules on the surface of neutrophils and endothelium.

- In **step 1**, the endothelial cells at sites of inflammation have increased expression of *E-selectin* and *P-selectin*.
- In **step 2**, neutrophils weakly bind to the endothelial selectins and roll along the surface.
- In **step 3**, neutrophils are stimulated by chemokines to express their integrins.
- In **step 4**, binding of the integrins to cellular adhesion molecules (ICAM-1 and VCAM-1) allows the neutrophils to firmly adhere to the endothelial cell.

Note

Selectins: weak binding; initiate rolling

Integrins: stable binding and adhesion

Table 3-1. Selectin and Integrin Distribution in the Endothelium and Leukocyte

	Endothelium	Leukocyte
Selectins	P-Selectin	Sialyl-Lewis X & PSGL-1
	E-Selectin	Sialyl-Lewis X & PSGL-1
	GlyCam-1/CD34	L-Selectin
Integrins	ICAM-1	LFA-1 & MAC-1
	VCAM-1	VLA-4

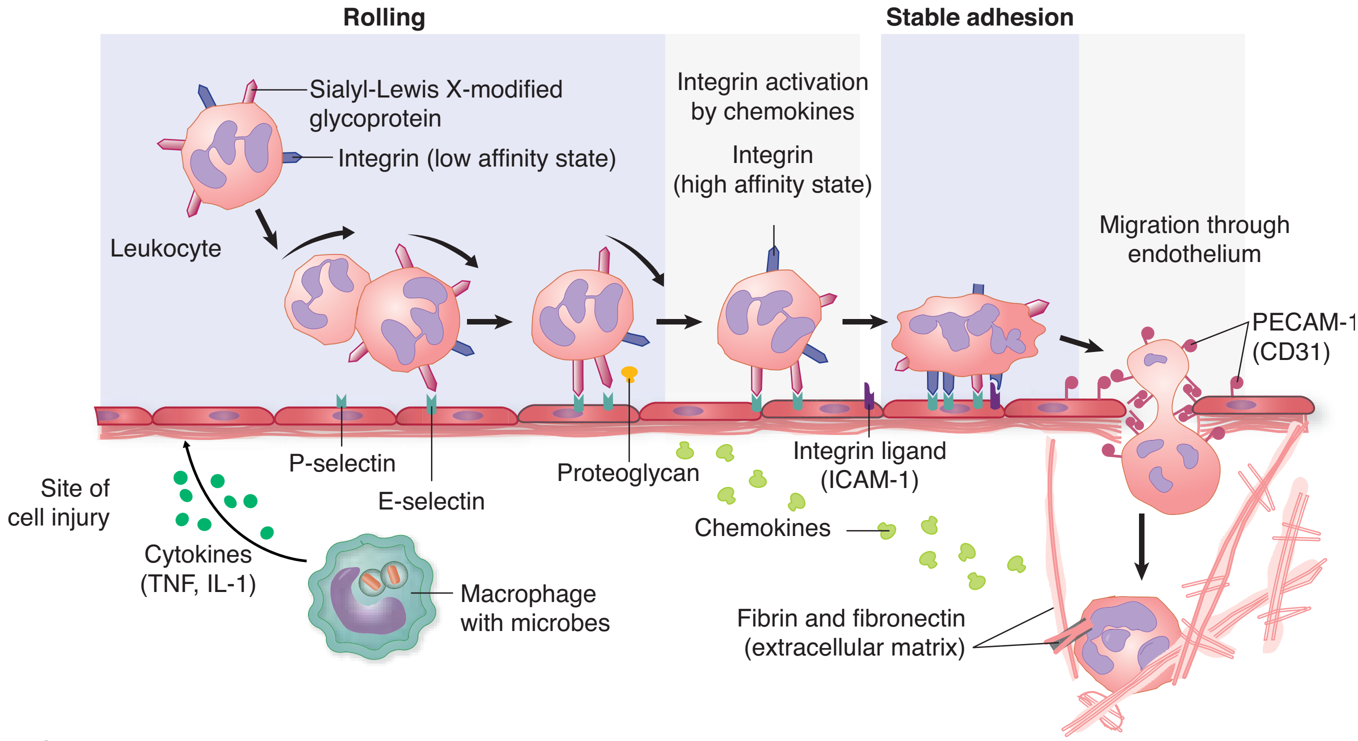


Figure 3-1. Adhesion and Migration

Modulation of adhesion molecules in inflammation occurs as follows. The fastest step involves redistribution of adhesion molecules to the surface; for example, P-selectin is normally present in the Weibel-Palade bodies of endothelial cells and can be mobilized to the cell surface by exposure to inflammatory mediators such as histamine and thrombin.

- Additionally, synthesis of adhesion molecules occurs. For example, proinflammatory cytokines IL-1 and TNF induce production of E-selectin, ICAM-1, and VCAM-1 in endothelial cells.
- There can also be increased binding affinity, as when chemotactic agents cause a conformational change in the leukocyte integrin LFA-1, which is converted to a high-affinity binding state.

Defects in adhesion can be seen in diabetes mellitus, corticosteroid use, acute alcohol intoxication, and leukocyte adhesion deficiency (autosomal recessive condition with recurrent bacterial infections).

In **emigration (diapedesis),** leukocytes emigrate from the vasculature (postcapillary venule) by extending pseudopods between the endothelial cells. They then move between the endothelial cells, migrating through the basement membrane toward the inflammatory stimulus.

Chemotaxis is the attraction of cells toward a chemical mediator that is released in the area of inflammation. Important chemotactic factors for neutrophils include bacterial products such as *N*-formyl-methionine and host derived molecules such as leukotriene B4 (LTB4), complement system product C5a, and α-chemokines (IL-8).

Clinical Correlate

Leukocyte adhesion deficiency type I

- Autosomal recessive
- Deficiency of β2 integrin subunit (CD18)
- Recurrent bacterial infection
- Delay in umbilical cord sloughing

Phagocytosis and degranulation. Opsonins coat microbes to enhance their detection and phagocytosis. Important opsonins include the Fc portion of IgG isotypes, complement system product C3b, and plasma proteins such as collectins (which bind to bacterial cell walls).

Engulfment occurs when the neutrophil sends out cytoplasmic processes that surround the bacteria. The bacteria are then internalized within a phagosome. The phagosome fuses with lysosomes (degranulation).

Defects in phagocytosis and degranulation include Chédiak-Higashi syndrome, an autosomal recessive condition characterized by neutropenia. The neutrophils have giant granules (lysosomes) and there is a defect in chemotaxis and degranulation.

Intracellular killing.

In oxygen-dependent killing, respiratory burst requires oxygen and NADPH oxidase and produces superoxide, hydroxyl radicals, and hydrogen peroxide. Myeloperoxidase requires hydrogen peroxide and halide (Cl^-) and produces HOCl (hypochlorous acid).

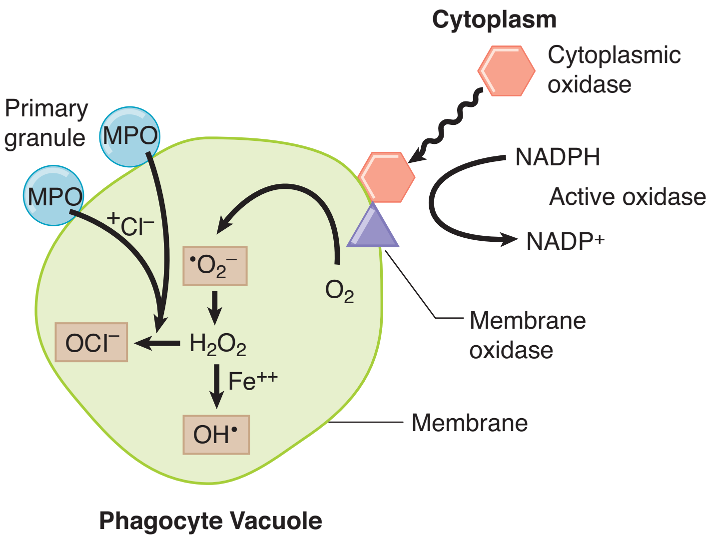
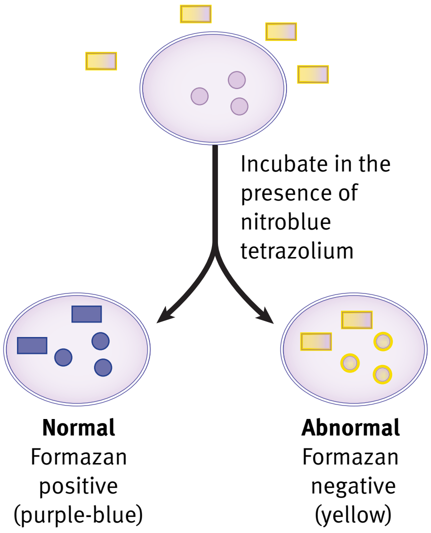


Figure 3-2. Oxygen-Dependent Killing

Oxygen-independent killing involves lysozyme, lactoferrin, acid hydrolases, bactericidal permeability increasing protein (BPI), and defensins.

Deficiencies of oxygen-dependent killing include:

- Chronic granulomatous disease of childhood can be X-linked or autosomal recessive. It is characterized by a deficiency of NADPH oxidase, lack of superoxide and hydrogen peroxide, and recurrent bacterial infections with catalase-positive organisms (*S. aureus*). The nitroblue tetrazolium test will be negative.
- Myeloperoxidase deficiency is an autosomal recessive condition characterized by infections with *Candida*. In contrast to chronic granulomatous disease, the nitroblue tetrazolium test will be positive.



Nitroblue Tetrazolium Reduction

Chemical Mediators of Inflammation

Vasoactive amines

- **Histamine** is produced by basophils, platelets, and mast cells. It causes vasodilation and increased vascular permeability. Triggers for release include IgE-mediated mast cell reactions, physical injury, anaphylatoxins (C3a and C5a), and cytokines (IL-1).
- **Serotonin** is produced by platelets and causes vasodilation and increased vascular permeability.

Kinin system

- Activated Hageman factor (factor XII) converts prekallikrein → kallikrein
- Kallikrein cleaves high molecular weight kininogen (HMWK) → bradykinin
- Effects of bradykinin include increased vascular permeability, pain, vasodilation, bronchoconstriction, and pain

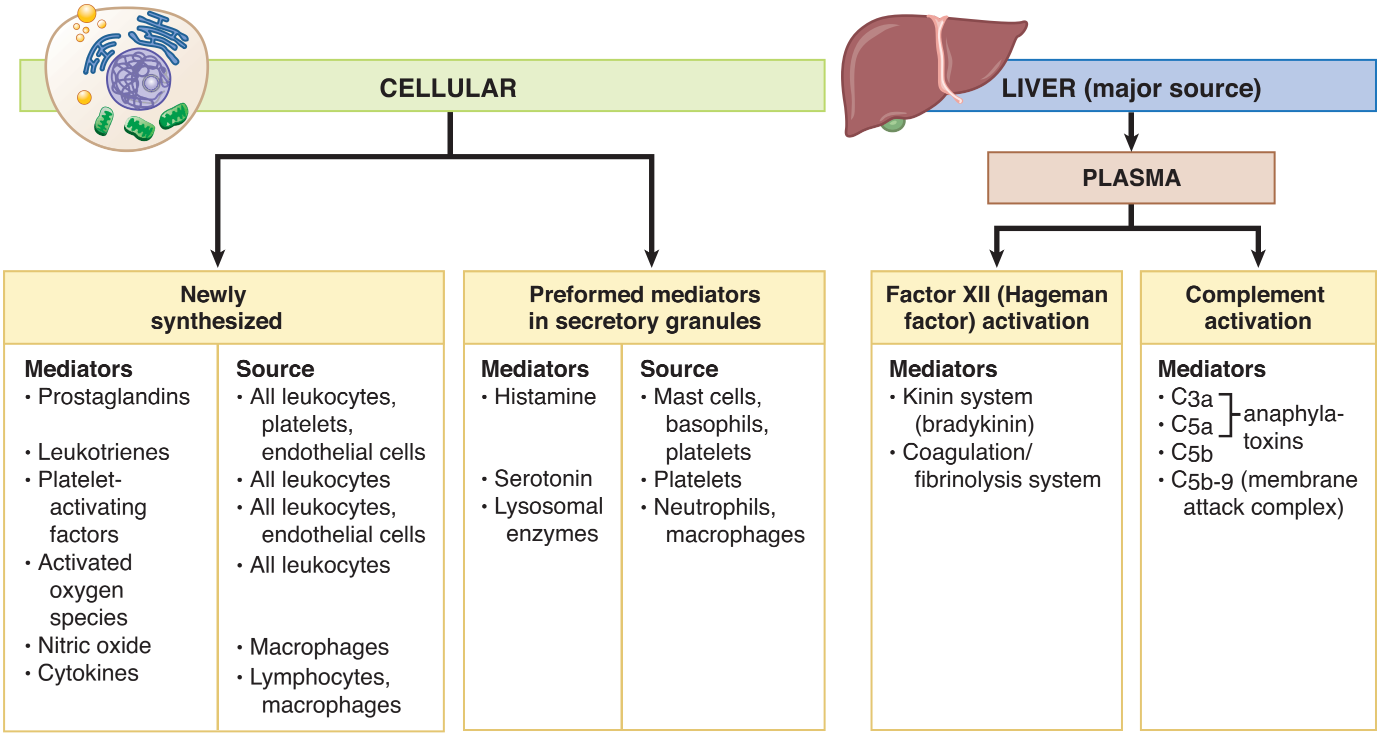


Figure 3-3. Sources of Chemical Mediators of Inflammation

Arachidonic acid products

- **Cyclooxygenase pathway**
  - Thromboxane A2 is produced by platelets and causes vasoconstriction and platelet aggregation.
  - Prostacyclin (PGI2) is produced by vascular endothelium and causes vasodilation and inhibition of platelet aggregation.
  - Prostaglandin E_2 causes pain.
  - Prostaglandins PGE2, PGD2, and PGF2 cause vasodilatation.

Note

Mediators of Pain

- Bradykinin
- Prostaglandins (E_2)

- **Lipoxygenase pathway**

  Leukotriene B4 (LTB4) causes neutrophil chemotaxis, while leukotriene C4, D4, E4 cause vasoconstriction. Lipoxins are antiinflammatory products which inhibit neutrophil chemotaxis.

Important products in the complement cascade include C5b-C9 (membrane attack complex), C3a, C5a (anaphylatoxins stimulate the release of histamine), C5a (leukocyte chemotactic factor), and C3b (opsonin for phagocytosis).

Cytokines

- IL-1 and TNF cause fever and induce acute phase reactants; enhance adhesion molecules; and stimulate and activate fibroblasts, endothelial cells, and neutrophils.
- IL-8 is a neutrophil chemoattractant produced by macrophages.

Note

Mediators of Fever

- Cytokines IL-1, IL-6, and TNF-α
- Prostaglandins

Four Outcomes of Acute Inflammation

- Complete resolution with regeneration
- Complete resolution with scarring
- Abscess formation
- Transition to chronic inflammation

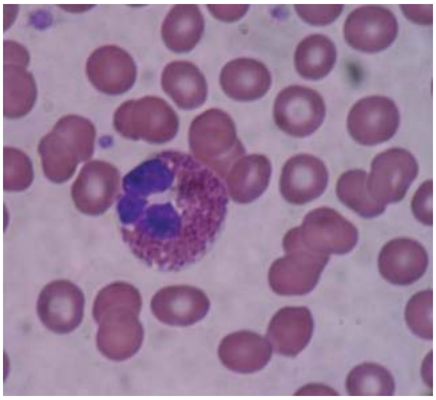
CHRONIC INFLAMMATION

Causes of chronic inflammation include the following:

- Following a bout of acute inflammation
- Persistent infections
- Infections with certain organisms, including viral infections, mycobacteria, parasitic infections, and fungal infections
- Autoimmune diseases
- Response to foreign material
- Response to malignant tumors

There are several **important cells** in chronic inflammation.

- **Macrophages** are derived from blood monocytes. Tissue-based macrophages (life span in connective tissue compartment is 60–120 days) are found in connective tissue (histiocyte), lung (pulmonary alveolar macrophages), liver (Kupffer cells), bone (osteoclasts), and brain (microglia). During inflammation circulating monocytes emigrate from the blood to the periphery and differentiate into macrophages.
  - Respond to chemotactic factors: C5a, MCP-1, MIP-1α, PDGF, TGF-β
  - Secrete a wide variety of active products (monokines)
  - May be modified into epithelioid cells in granulomatous processes
- **Lymphocytes** include B cells and plasma cells, as well as T cells. Lymphotaxin is the lymphocyte chemokine.



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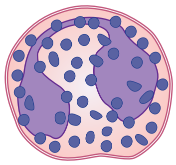
Bilobed nucleus, large granules (pink)

Eosinophil

- **Eosinophils** play an important role in parasitic infections and IgE-mediated allergic reactions. The eosinophilic chemokine is eotaxin. Eosinophil granules contain major basic protein, which is toxic to parasites.
- **Basophils** contain similar chemical mediators as mast cells in their granules. Mast cells are present in high numbers in the lung and skin. Both basophils and mast cells play an important role in IgE-mediated reactions (allergies and anaphylaxis) and can release histamine.



Bilobed nucleus, large granules (blue)

Basophil

Chronic granulomatous inflammation is a specialized form of chronic inflammation characterized by small aggregates of modified macrophages (epithelioid cells and multinucleated giant cells) usually populated by CD4+ Th1 lymphocytes.

Composition of a granuloma is as follows:

- Epithelioid cells, located centrally, form when IFN-γ transforms macrophages to epithelioid cells. They are enlarged cells with abundant pink cytoplasm.
- Multinucleated giant cells, located centrally, are formed by the fusion of epithelioid cells. Types include Langhans-type giant cell (peripheral arrangement of nuclei) and foreign body type giant cell (haphazard arrangement of nuclei).
- Lymphocytes and plasma cells are present at the periphery.
- Central necrosis occurs in granulomata due to excessive enzymatic breakdown and is commonly seen in *Mycobacterium tuberculosis* infection as well as fungal infections and a few bacterial infections. Because of the public health risk of tuberculosis, necrotizing granulomas should be considered tuberculosis until proven otherwise.

Clinical Correlate

Patients who are to be placed on tumor necrosis factor (TNF) inhibitors such as infliximab must undergo a PPD test before starting therapy. The PPD checks for latent tuberculosis infection, which can be reactivated by the TNF alpha inhibitor.

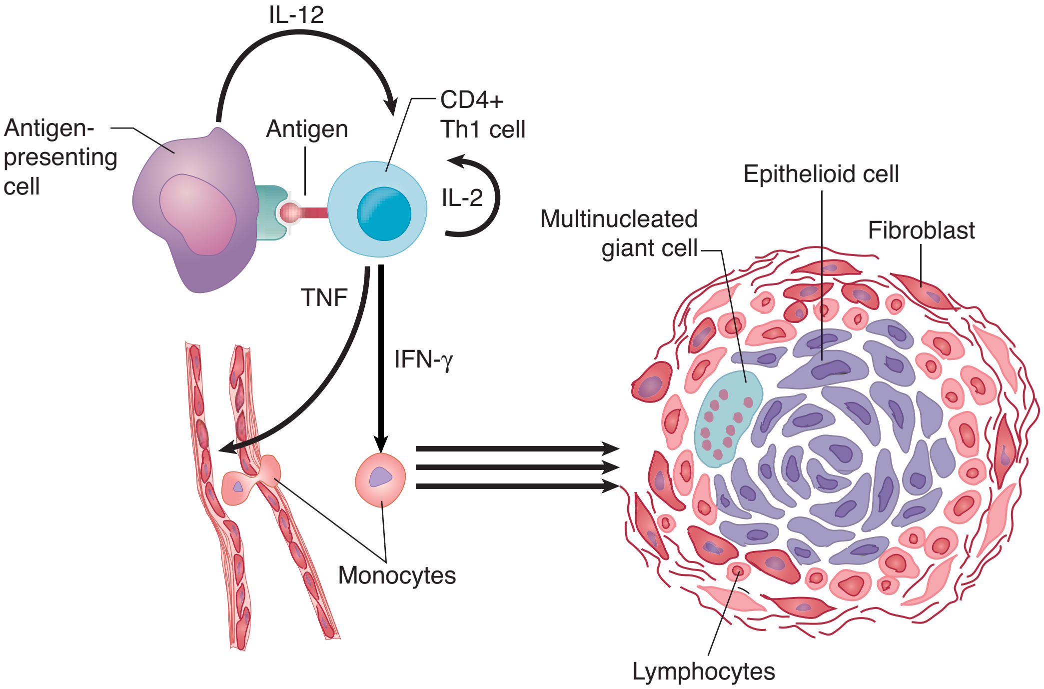


Figure 3-4. Granuloma Formation

Granulomatous diseases include tuberculosis (caseating granulomas), cat-scratch fever, syphilis, leprosy, fungal infections (e.g., coccidioidomycosis), parasitic infections (e.g., schistosomiasis), foreign bodies, beryllium, and sarcoidosis.

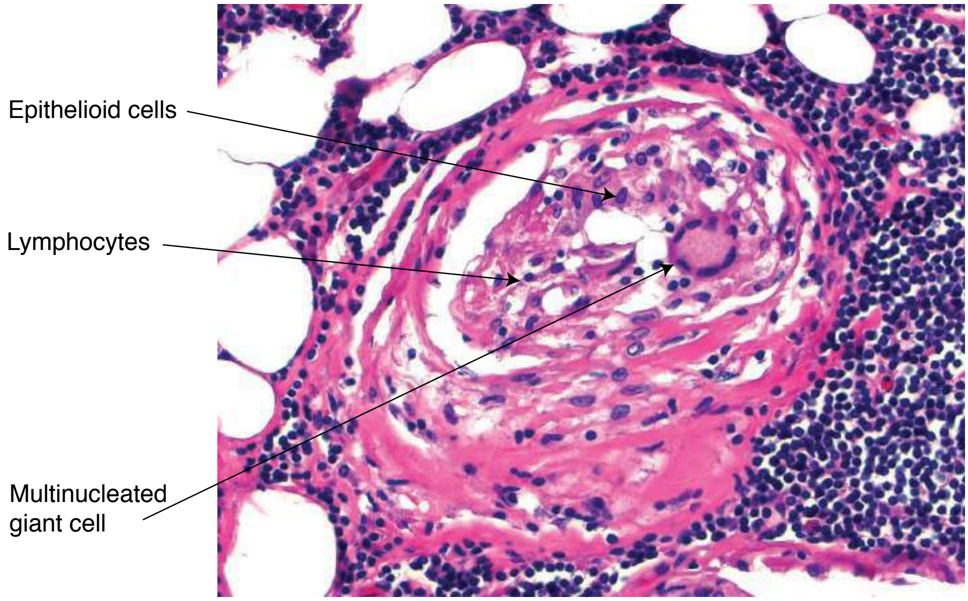


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Figure 3-5. A Granuloma Is Seen in the Large, Poorly Circumscribed Nodule in the Center of the Field

TISSUE RESPONSES TO INFECTIOUS AGENTS

Infectious diseases are very prevalent worldwide and are a major cause of morbidity and mortality. Infectious agents tend to have **tropism** for specific tissues and organs.

There are **6 major histologic patterns:**

- **Exudative inflammation** is acute inflammatory response with neutrophils. Examples include bacterial meningitis, bronchopneumonia, and abscess.
- **Necrotizing inflammation** occurs when a virulent organism produces severe tissue damage and extensive cell death. Examples include necrotizing fasciitis and necrotizing pharyngitis.
- **Granulomatous inflammation.** Granulomatous response predominates with slow-growing organisms such as mycobacteria, fungi, and parasites.
- **Interstitial inflammation** is a diffuse mononuclear interstitial infiltrate that is a common response to viral infectious agents. Examples include myocarditis (Coxsackie virus) and viral hepatitis.
- **Cytopathic/cytoproliferative** inflammation refers to inflammation in which the infected/injured cell is altered. The changes may include intranuclear/cytoplasmic inclusions (cytomegalic inclusion disease, rabies [Negri body]); syncytia formation (respiratory syncytial virus and herpes virus); and apoptosis (Councilman body in viral hepatitis).
- **No inflammation.** An inflammatory response to microbes cannot occur in severely Interleukin-1, inflammation mediation immunosuppressed individuals due to primary immunodeficiencies or acquired immunodeficient states (e.g., AIDS).

Tissue Repair 4

Learning Objectives

- ❏ Demonstrate understanding of regeneration and healing
- ❏ Answer questions about aberrations in wound healing

REGENERATION AND HEALING

Regeneration and healing of damaged cells and tissues start almost as soon as the inflammatory process begins. Tissue repair involves 5 overlapping processes:

- Hemostasis (coagulation, platelets)
- Inflammation (neutrophils, macrophages, lymphocytes, mast cells)
- Regeneration (stem cells and differentiated cells)
- Fibrosis (macrophages, granulation tissue [fibroblasts, angiogenesis], type III collagen)
- Remodeling (macrophages, fibroblasts, converting collagen III to I)

The extracellular matrix (ECM) is an important tissue scaffold with 2 forms, the interstitial matrix and the basement membrane (type IV collagen and laminin). There are 3 ECM components:

- Collagens and elastins
- Gels (proteoglycans and hyaluronan)
- Glycoproteins and cell adhesion molecules

Different tissues have different **regenerative** capacities.

- **Labile cells** (primarily stem cells) regenerate throughout life. Examples include surface epithelial cells (skin and mucosal lining cells), hematopoietic cells, stem cells, etc.
- **Stable cells** (stem cells and differentiated cells) replicate at a low level throughout life and have the capacity to divide if stimulated by some initiating event. Examples include hepatocytes, proximal tubule cells, endothelium, etc.
- **Permanent cells** (few stem cells and/or differentiated cells with the capacity to replicate) have a very low level of replicative capacity. Examples include neurons and cardiac muscle.

Scar formation occurs in a series of steps when repair cannot be brought about by regeneration.

- First, angiogenesis is promoted by vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF) family of growth factors.

- Next, platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF-2), and transforming growth factor β (TGF-β) drive fibroblast activation for the formation of granulation tissue.
- Then, TGF-β, PDGF, and FGF drive ECM deposition. Cytokines IL-1 and IL-13 stimulate collagen production for scar formation.

Types of Wound Healing

Primary union (healing by first intention) occurs when wounds are closed physically with sutures, metal staples, dermal adhesive, etc.

Secondary union (healing by secondary intention) occurs when wounds are allowed to heal by wound contraction and is mediated by myofibroblasts at the edge of the wound.

Note

Clinicians make decisions about wound healing techniques based on clinical information and the size of the tissue defect.

Repair in specific organs occurs as follows:

- **Liver:** Mild injury is repaired by regeneration of hepatocytes, sometimes with restoration to normal pathology. Severe or persistent injury causes formation of regenerative nodules that may be surrounded by fibrosis, leading to hepatic cirrhosis.
- In the **brain**, neurons do not regenerate, but microglia remove debris and astrocytes proliferate, causing gliosis.
- Damaged **heart muscle** cannot regenerate, so the heart heals by fibrosis.
- In the **lung**, type II pneumocytes replace type I pneumocytes after injury.
- In **peripheral nerves**, the distal part of the axon degenerates while the proximal part regrows slowly, using axonal sprouts to follow Schwann cells to the muscle.

ABERRATIONS IN WOUND HEALING

- **Delayed wound healing** may be seen in wounds complicated by foreign bodies, infection, ischemia, diabetes, malnutrition, scurvy, etc.
- **Hypertrophic scar** results in a prominent scar that is localized to the wound, due to excess production of granulation tissue and collagen. It is common in burn patients.
- **Keloid formation** is a genetic predisposition that is common in African Americans. It tends to affect the earlobes, face, neck, sternum, and forearms, and it may produce large tumor-like scars extending beyond the injury site. There is excess production of collagen that is predominantly type III.

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Figure 4-1. Keloid on Posterior Surface of Ear (Auricle)

Circulatory Pathology 5

Learning Objectives

- ❑ Use knowledge of edema, hemostasis, and bleeding disorders to solve problems
- ❑ Answer questions about thrombosis, embolism, and infarction
- ❑ Solve problems concerning shock

EDEMA

Edema is the presence of excess fluid in the intercellular space. It has many causes.

- **Increased hydrostatic pressure** causes edema in congestive heart failure (generalized edema), portal hypertension, renal retention of salt and water, and venous thrombosis (local edema).
- **Hypoalbuminemia and decreased colloid osmotic pressure** cause edema in liver disease, nephrotic syndrome, and protein deficiency (e.g., kwashiorkor).
- **Lymphatic obstruction** (lymphedema) causes edema in tumor, following surgical removal of lymph node drainage, and in parasitic infestation (filariasis → elephantiasis).
- **Increased endothelial permeability** causes edema in inflammation, type I hypersensitivity reactions, and with some drugs (e.g., bleomycin, heroin, etc.).
- **Increased interstitial sodium** causes edema when there is increased sodium intake, primary hyperaldosteronism, and renal failure.
- Specialized forms of tissue swelling due to **increased extracellular glycosaminoglycans** also occur, notably in pretibial myxedema and exophthalmos (Graves disease).

Anasarca is severe generalized edema. **Effusion** is fluid within the body cavities.

Types of Edema Fluid

- **Transudate** is edema fluid with low protein content.
- **Exudate** is edema fluid with high protein content and cells. Types of exudates include purulent (pus), fibrinous, eosinophilic, and hemorrhagic.
- **Lymphedema** related to lymphatic obstruction leads to accumulation of protein-rich fluid which produces a non-pitting edema.
- **Glycosaminoglycan-rich** edema fluid shows increased hyaluronic acid and chondroitin sulfate, and causes myxedema.

Note

Edema can be localized or generalized, depending on the etiology and severity.

Active hyperemia versus congestion (passive hyperemia): an excessive amount of blood in a tissue or organ can accumulate secondary to vasodilatation (active, e.g., inflammation) or diminished venous outflow (passive, e.g., hepatic congestion).

HEMOSTASIS AND BLEEDING DISORDERS

Hemostasis is a sequence of events leading to the cessation of bleeding by the formation of a stable fibrin-platelet hemostatic plug. It involves interactions between the vascular wall, platelets, and the coagulation system.

Note

Clotting is a balance between 2 opposing forces: those favoring the formation of a stable thrombus versus those factors causing fibrinolysis of the clot.

Vascular Wall Injury

Transient vasoconstriction is mediated by endothelin-1. Thrombogenic factors include a variety of processes:

- Changes in blood flow cause turbulence and stasis favor clot formation.
- Release of tissue factor from injured cells activates factor VII (extrinsic pathway).
- Exposure of thrombogenic subendothelial collagen activates factor XII (intrinsic pathway).
- Release of von Willebrand factor (vWF) binds to exposed collagen and facilitates platelet adhesion.
- Decreased endothelial synthesis of antithrombogenic substances (prostacyclin, nitric oxide [NO_2], tissue plasminogen activator, and thrombomodulin)

Bridge to Pharmacology

Aspirin irreversibly acetylates cyclooxygenase, preventing platelet production of thromboxane A2.

Platelets

Platelets are derived from megakaryocytes in the bone marrow. They form a thrombus through a series of steps.

- **Step 1: Platelet adhesion** occurs when vWF adheres to subendothelial collagen and then platelets adhere to vWF by glycoprotein Ib.
- **Step 2: Platelet activation** occurs when platelets undergo a shape change and degranulation occurs. Platelets synthesize thromboxane A2. Platelets also show membrane expression of the phospholipid complex, which is an important substrate for the coagulation cascade.
- **Step 3: Platelet aggregation** occurs when additional platelets are recruited from the bloodstream. ADP and thromboxane A2 are potent mediators of aggregation. Platelets bind to each other by binding to fibrinogen using GPIIb-IIIa.

Laboratory tests for platelets include platelet count (normal 150,000–400,000 mm^3) and platelet aggregometry.

Bernard-Soulier syndrome and Glanzmann thrombasthenia present as mucocutaneous bleeding in childhood.

Note

Bernard-Soulier Syndrome

- Usually autosomal recessive
- Defects of the GPIb IX-V
- Defective platelet adhesion

Table 5-1. Contents of Platelet Alpha Granules and Dense Bodies

Alpha Granules	Dense Bodies
• Fibrinogen	• ADP (potent platelet aggregator)
• Fibronectin	• Calcium
• Factor V and vWF	• Histamine and serotonin
• Platelet factor 4	• Epinephrine
• Platelet-derived growth factor (PDGF)	

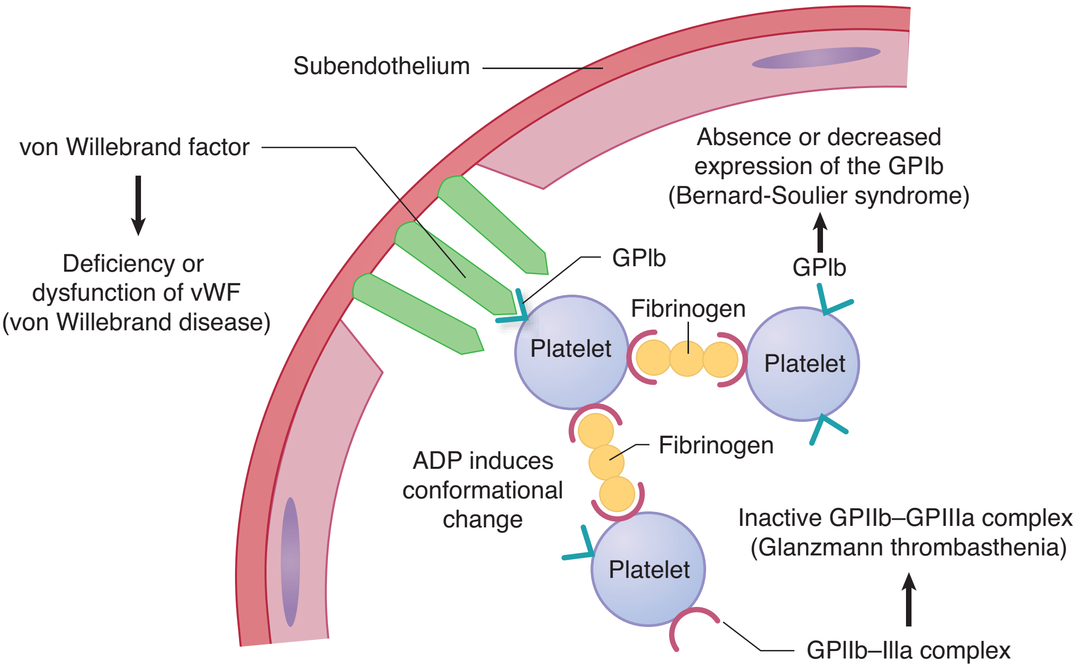

Figure 5-1. Platelet Aggregation

Table 5-2. Common Platelet Disorders

Thrombocytopenia	Qualitative Defects
Decreased production • Aplastic anemia (drugs, virus, etc.) • Tumor	• von Willebrand disease • Bernard-Soulier syndrome • Glanzmann thrombasthenia • Drugs (aspirin) • Uremia
Increased destruction • Immune thrombocytopenia (ITP) • Thrombotic thrombocytopenic purpura (TTP) • Disseminated intravascular coagulation (DIC) • Hypersplenism	

Note

Glanzmann Thrombasthenia

- Autosomal recessive
- Defective GPIIb-IIIa receptor
- Defective platelet aggregation

Immune thrombocytopenia purpura (ITP) is an immune-mediated attack (usually IgG antiplatelet antibodies) against platelets leading to decreased platelets (thrombocytopenia) which result in petechiae, purpura (bruises), and a bleeding diathesis (e.g., hematomas).

The etiology involves antiplatelet antibodies against platelet antigens such as GPIIb-IIIa and GPIb-IX (type II hypersensitivity reaction). The antibodies are made in the spleen, and the platelets are destroyed peripherally in the spleen by macrophages, which have Fc receptors that bind IgG-coated platelets.

Forms of ITP include:

- Acute ITP, seen in children following a viral infection and is a self-limited disorder.
- Chronic ITP, usually seen in women in their childbearing years and may be the first manifestation of systemic lupus erythematosus (SLE). Clinically, it is characterized by petechiae, ecchymoses, menorrhagia, and nosebleeds.

Lab studies usually show decreased platelet count and prolonged bleeding time but normal prothrombin time and partial thromboplastin time. Peripheral blood smear shows thrombocytopenia with enlarged immature platelets (megathrombocytes). Bone marrow biopsy shows increased numbers of megakaryocytes with immature forms.

Treatment is corticosteroids, which decrease antibody production; immunoglobulin therapy, which floods Fc receptors on splenic macrophages; and/or splenectomy, which removes the site of platelet destruction and antibody production.

Thrombotic thrombocytopenic purpura (TTP) is a rare disorder of hemostasis in which there is widespread intravascular formation of fibrin-platelet thrombi. It is sometimes associated with an acquired or inherited deficiency of the enzyme ADAMTS13, responsible for cleaving large multimers of von Willebrand factor.

Clinically, TTP most often affects adult women. The inclusion criteria are microangiopathic hemolytic anemia and thrombocytopenia, with or without renal failure or neurologic abnormalities. Pathology includes widespread formation of platelet thrombi with fibrin (hyaline thrombi) leading to intravascular hemolysis (thrombotic microangiopathy).

Lab studies typically show decreased platelet count and prolonged bleeding time but normal prothrombin time and partial thromboplastin time. Peripheral blood smear shows thrombocytopenia, schistocytes, and reticulocytosis. Treatment is plasma exchange.

Hemolytic uremic syndrome (HUS) is a form of thrombotic microangiopathy due to endothelial cell damage. It occurs mostly in children, typically after a gastroenteritis (typically due to Shiga toxin-producing *E. coli* 0157:H7).

Typical HUS presents with abdominal pain, diarrhea (an atypical variant is diarrhea-negative), microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. Renal involvement is seen more commonly than in TTP. The kidney shows fibrin thrombi in the glomeruli. Renal glomerular endothelial cells are targeted by the bacterial toxin. Glomerular scarring may ensue.

Treatment is supportive (fluid management, dialysis, erythrocyte transfusions); plasma exchange is only used for atypical cases.

Coagulation factors. The majority of the clotting factors are produced by the liver. The factors are proenzymes that must be converted to the active form. Some conversions occur on a phospholipid surface, and some conversions require calcium.

- The **intrinsic coagulation pathway** is activated by the contact factors, which include contact with subendothelial collagen, high molecular weight kininogen (HMWK), and kallikrein.
- The **extrinsic coagulation pathway** is activated by the release of tissue factor.

Note

- Patients on warfarin therapy should be monitored using prothrombin time (WEPT = warfarin, extrinsic PT).
- Patients on heparin therapy should be monitored using partial thromboplastin time (HIPTT = heparin, intrinsic PTT).

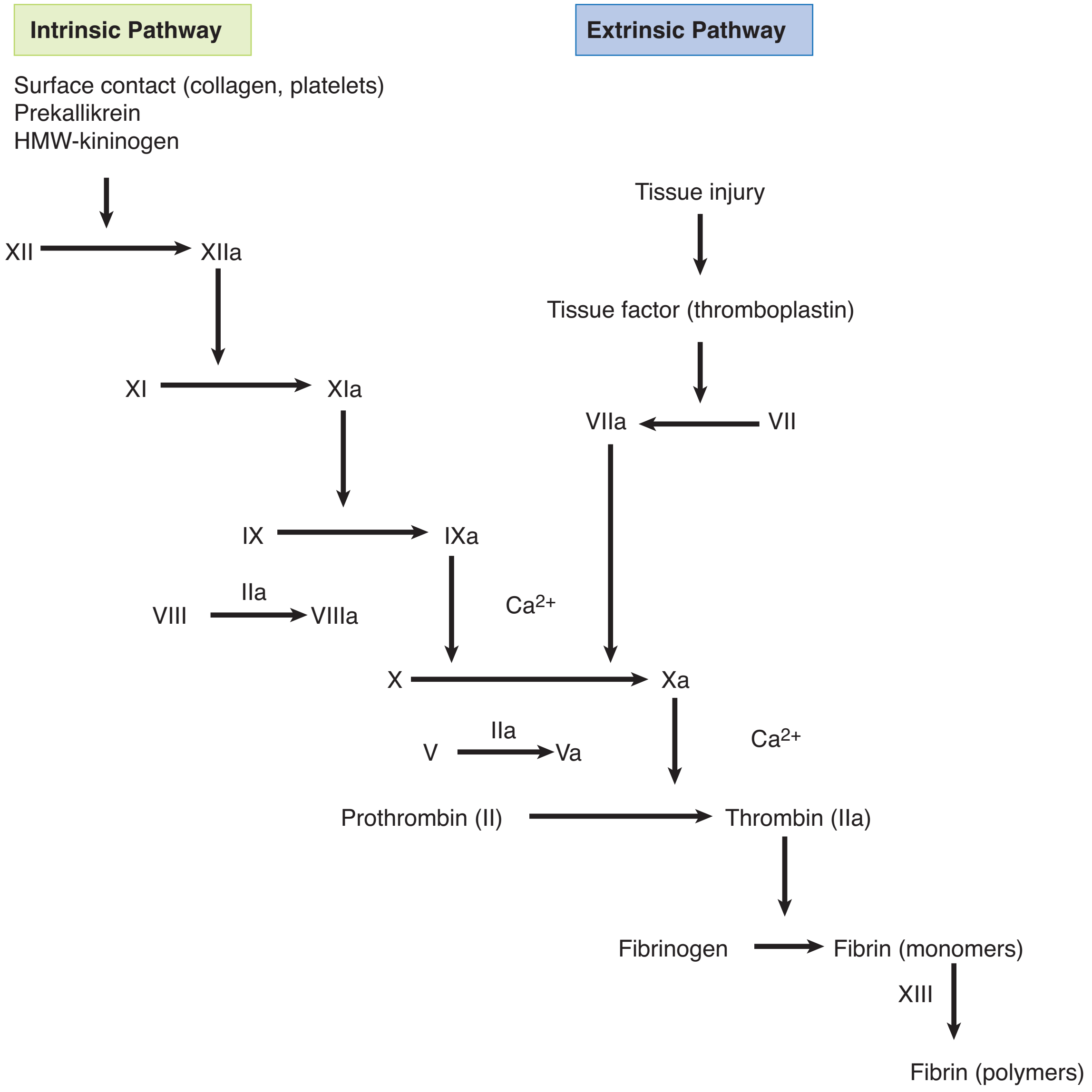

Figure 5-2. Coagulation Cascade

Lab tests for coagulation include the following:

- Prothrombin time (PT), which tests the extrinsic and common coagulation pathways (more specifically, it tests factors VII, X, V, prothrombin, and fibrinogen). The international normalized ratio (INR) standardizes the PT

test so that results throughout the world can be compared. A longer time means blood takes longer to clot.

- Partial thromboplastin time (PTT), which tests the intrinsic and common coagulation pathways (more specifically, it tests factors XII, XI, IX, VIII, X, V, prothrombin, and fibrinogen).
- Thrombin time (TT), which tests for adequate fibrinogen levels.
- Fibrin degradation products (FDP), which tests the fibrinolytic system (increased with DIC).

Hemophilia A (classic hemophilia) is an X-linked recessive condition resulting from a deficiency of factor VIII. Clinically, hemophilia A predominately affects males. Symptoms vary depending on the degree of deficiency.

- Newborns may develop bleeding at the time of circumcision.
- Other problems include spontaneous hemorrhage into joints (hemarthrosis), easy bruising and hematoma formation after minor trauma, and severe prolonged bleeding after surgery or lacerations.

Laboratory studies typically show normal platelet count and normal bleeding time, normal PT and prolonged PTT. Treatment is factor VIII concentrate.

Hemophilia B (Christmas disease) is an X-linked recessive condition resulting from a deficiency of factor IX that is clinically identical to hemophilia A. Treatment is recombinant factor IX.

Acquired coagulopathies include vitamin K deficiency (decreased synthesis of factors II, VII, IX, X, and protein C & S) and liver disease (decreased synthesis of virtually all clotting factors).

Note

Von Willebrand disease is the most common inherited bleeding disorder.

Von Willebrand disease is an autosomal dominant bleeding disorder characterized by a deficiency or qualitative defect in von Willebrand factor. vWF is normally produced by endothelial cells and megakaryocytes. Clinical features include spontaneous bleeding from mucous membranes, prolonged bleeding from wounds, and menorrhagia in young females. Hemarthrosis is uncommon.

Lab studies show normal platelet count, a prolonged bleeding time, normal PT, and often prolonged PTT. Abnormal platelet response to ristocetin (adhesion defect) is an important diagnostic test. Treatment for mild classic cases (type I) is desmopressin (an antidiuretic hormone analog), which releases vWF from Weibel-Palade bodies of endothelial cells.

Disseminated intravascular coagulation (DIC) is always secondary to another disorder. Causes are diverse.

- Obstetric complications can cause DIC because placental tissue factor activates clotting.
- Gram-negative sepsis can cause DIC because tumor necrosis factor activates clotting.
- Microorganisms (especially meningococcus and rickettsiae)
- AML M3 (cytoplasmic granules in neoplastic promyelocytes activate clotting)
- Adenocarcinomas (mucin activates clotting)

DIC causes widespread microthrombi with consumption of platelets and clotting factors, causing hemorrhage. Laboratory studies show decreased platelet count, prolonged PT/PTT, decreased fibrinogen, and elevated fibrin split products (D dimers). Treat the underlying disorder.

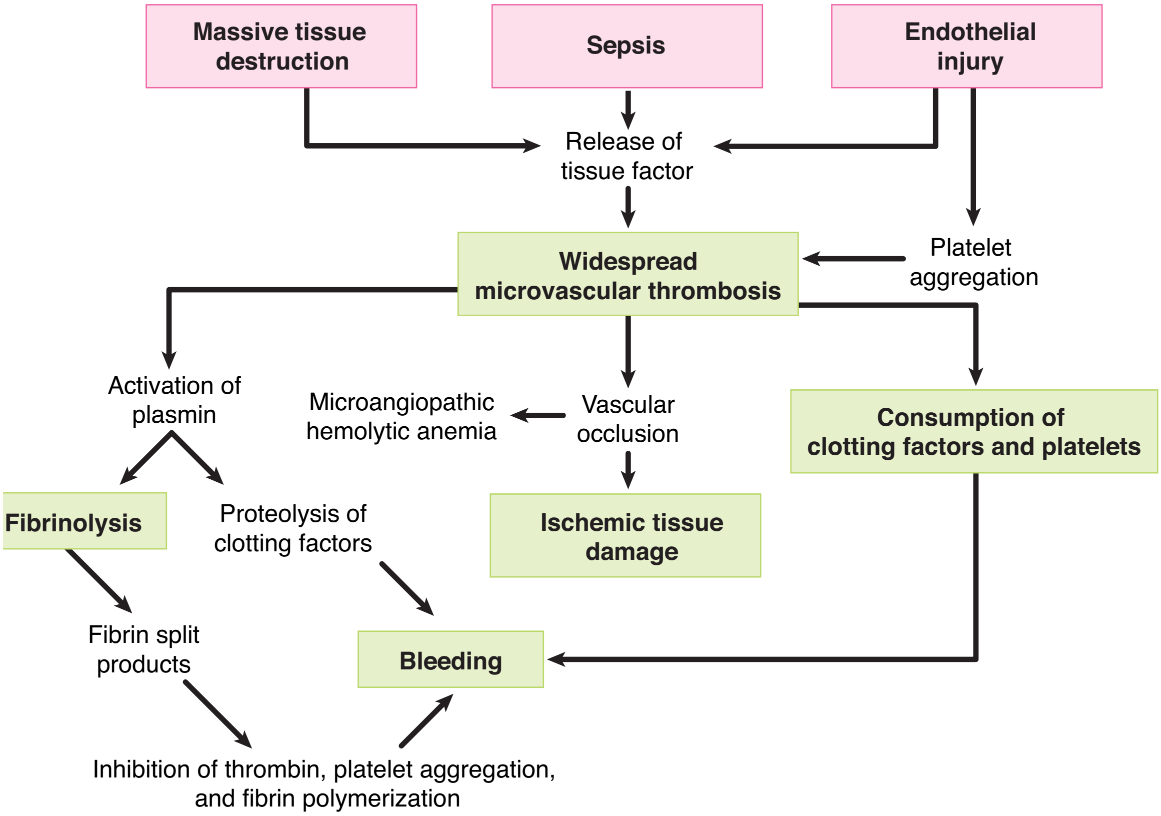

Figure 5-3. Disseminated Intravascular Coagulation

THROMBOSIS

Thrombosis is the pathologic formation of an intravascular fibrin-platelet thrombus during life. Factors involved in thrombus formation (**Virchow's triad**) include:

- Endothelial injury due to atherosclerosis, vasculitis, or many other causes
- Alterations in laminar blood flow predisposing for DIC occur with stasis of blood (e.g., immobilization); turbulence (e.g., aneurysms); and hyperviscosity of blood (e.g., polycythemia vera)
- Hypercoagulability of blood can be seen with clotting disorders (factor V Leiden; deficiency of antithrombin III, protein C, or protein S); tissue injury (postoperative and trauma); neoplasia; nephrotic syndrome; advanced age; pregnancy; and oral contraceptives (estrogen increases synthetic activity of the liver, including clotting factors)

Table 5-3. Comparison of a Thrombus with a Blood Clot

	Thrombus	Blood Clot
Location	Intravascular	Extravascular or intravascular (postmortem)
Composition	Platelets	Lacks platelets
	Fibrin	Fibrin
	RBCs and WBCs	RBCs and WBCs
Lines of Zahn	Present	Absent
Shape	Has shape	Lacks shape

Common locations of thrombus formation include coronary and cerebral arteries; heart chambers in atrial fibrillation or post-MI (mural thrombus); aortic aneurysms; heart valves (vegetations); and deep leg veins (DVTs).

Outcomes of thrombosis include vascular occlusion and infarctions; embolism; thrombolysis; and organization and recanalization.

EMBOLISM

An embolism is any intravascular mass that has been carried down the bloodstream from its site of origin, resulting in the occlusion of a vessel. There are many types of emboli:

- **Thromboemboli:** most common (98%)
- Atheromatous emboli (severe atherosclerosis)
- Fat emboli (bone fractures and soft tissue trauma)
- Bone marrow emboli (bone fractures and cardiopulmonary resuscitation [CPR])
- Gas emboli cause decompression sickness (“the bends” and caisson disease) when rapid ascent results in nitrogen gas bubbles in the blood vessels
- Amniotic fluid emboli are a complication of labor that may result in DIC; fetal squamous cells are seen in the maternal pulmonary vessels
- Tumor emboli (metastasis)
- Talc emboli (IV drug abuse)
- Bacterial/septic emboli (infectious endocarditis)

Bridge to Anatomy

The dual blood supply to the lungs is from the pulmonary artery and the bronchial arteries.

Clinical Correlate

The classic presentation of pulmonary embolism, which occurs in <20% of patients, includes hemoptysis, dyspnea, and chest pain.

Pulmonary emboli (PE) are often clinically silent and are the most commonly missed diagnosis in hospitalized patients. They are found in almost 50% of all hospital autopsies. Most PE (95%) arise from deep leg vein thrombosis (DVT) in the leg; other sources include the right side of the heart and the pelvic venous plexuses of the prostate and uterus.

Diagnosis of a PE can be established when V/Q lung shows a scan V/Q mismatch. Doppler ultrasound of the leg veins can be used to detect a DVT. Additionally, plasma D-dimer ELISA test is elevated.

Most cases are clinically silent and resolve.

Infarction is more common in patients with cardiopulmonary compromise. Symptoms include shortness of breath, hemoptysis, pleuritic chest pain, and pleural effusion. On gross examination there is typically a hemorrhagic wedge-shaped infarct. The infarction heals by regeneration or scar formation.

- Sudden death can occur when large emboli lodge in the bifurcation (saddle embolus) or large pulmonary artery branches.
- Chronic secondary pulmonary hypertension is caused by recurrent PEs, which increase pulmonary resistance and lead to secondary pulmonary hypertension.

INFARCTION

Infarction is a localized area of necrosis secondary to ischemia. Common sites of infarction include heart, brain, lungs, intestines, kidneys. Infarcts have multiple causes.

- Most infarcts (99%) result from thrombotic or embolic occlusion of an artery or vein.
- Less common causes include vasospasm and torsion of arteries and veins (e.g., volvulus, ovarian, and testicular torsion).

On gross examination infarctions typically have a wedge shape, with the apex of the wedge tending to point to the occlusion.

- **Anemic infarcts (pale or white color)** occur in solid organs with a single blood supply such as the spleen, kidney, and heart.
- **Hemorrhagic infarcts (red color)** occur in organs with a dual blood supply or collateral circulation, such as the lung and intestines, and can also occur with venous occlusion (e.g., testicular torsion).

Microscopic pathology of infarction can show either coagulative necrosis (most organs) or liquefactive necrosis (brain). The general sequence of tissue changes after infarction is as follows:

ischemia → coagulative necrosis → inflammation → granulation tissue → fibrous scar

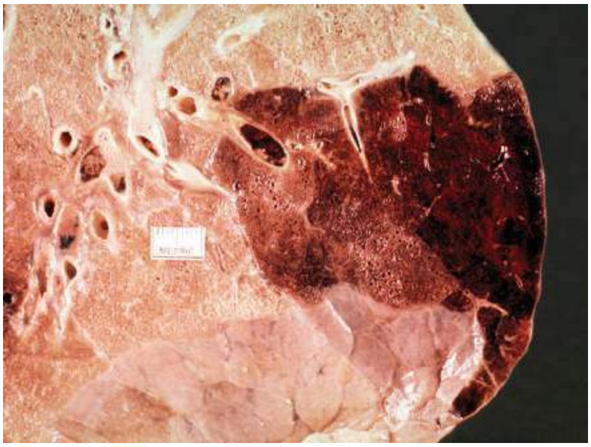

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Figure 5-4. Wedge-Shaped Pulmonary Infarction

SHOCK

Shock is characterized by vascular collapse and widespread hypoperfusion of cells and tissue due to reduced blood volume, cardiac output, or vascular tone. The cellular injury is initially reversible; if the hypoxia persists, the cellular injury becomes irreversible, leading to the death of cells and the patient.

Major Causes of Shock

- **Cardiogenic shock** (pump failure) can be due to myocardial infarction, cardiac arrhythmias, pulmonary embolism, and cardiac tamponade.
- **Hypovolemic shock** (reduced blood volume) can be due to hemorrhage, fluid loss secondary to severe burns, and severe dehydration.
- **Septic shock** (viral or bacterial infection) causes cytokines to trigger vasodilatation and hypotension, acute respiratory distress syndrome (ARDS), DIC, and multiple organ dysfunction syndrome. Mortality rate is 20%.
- **Neurogenic shock** (generalized vasodilatation) can be seen with anesthesia and brain or spinal cord injury.
- **Anaphylactic shock** (generalized vasodilatation) is a type I hypersensitivity reaction.

Stages of Shock

The stages of shock are arbitrarily defined as follows.

- **Stage I: compensation**

  Perfusion to vital organs is maintained by reflex mechanisms. Compensation is characterized by increased sympathetic tone, release of catecholamines, and activation of the renin-angiotensin system.

- **Stage II: decompensation**

  There is a progressive decrease in tissue perfusion, leading to potentially reversible tissue injury with development of a metabolic (lactic) acidosis, electrolyte imbalances, and renal insufficiency.

- **Stage III: irreversible tissue injury and organ failure**

  This ultimately results in death.

The organs show various manifestations of shock:

- Kidneys show fibrin thrombi in glomeruli and ultimately, acute tubular failure ensues, which causes oliguria and electrolyte imbalances.
- Lungs undergo diffuse alveolar damage (“shock lung”).
- Intestines show superficial mucosal ischemic necrosis and hemorrhages, and with prolonged injury, bacteremia may ensue.

Genetic Disorders 6

Learning Objectives

- ❏ Answer questions about disorders involving an extra autosome and chromosomal deletions
- ❏ Demonstrate understanding of Mendelian disorders, autosomal recessive/dominant, and x-linked recessive/dominant conditions
- ❏ Solve problems concerning triplet repeat mutations
- ❏ Explain information related to mitochondrial DNA disorders and multifactorial inheritance

DISORDERS INVOLVING AN EXTRA AUTOSOME

Down syndrome (trisomy 21).

The most common **karyotype** is 47, XX, +21. Down syndrome is the **most common of the chromosomal disorders**. The risk increases with maternal age to an incidence of 1 in 25 live births in women age ≥45. The pathogenesis involves meiotic nondisjunction (95%), Robertsonian translocation (4%), or mosaicism due to mitotic nondisjunction during embryogenesis (1%).

Clinical findings can include intellectual disability; mongoloid facial features (flat face, low-bridged nose, and epicanthal folds); Brushfield spots (speckled appearance of the iris); muscular hypotonia; broad short neck; palmar (simian) crease; and congenital heart defects. Endocardial cushion defect, if present, leads to the formation of an atrioventricular canal (a common connection between all 4 chambers of the heart). Additional clinical problems that can develop include duodenal atresia ("double-bubble" sign); Hirschsprung disease; increased risk (15–20 fold) of acute lymphoblastic leukemia (ALL); and Alzheimer disease (by age 40 virtually all will develop Alzheimer disease).

Prenatal tests include maternal serum tests, ultrasonography, amniocentesis, and chorionic villus sampling.

Median life expectancy is 47 years.

Note

Robertsonian Translocation

Defined as a translocation involving 2 acrocentric chromosomes with the break points occurring close to the centromeres. This results in an extremely large chromosome and a tiny one, which is typically lost.

Note

Mosaicism is defined as the presence of ≥2 populations of cells within an individual.

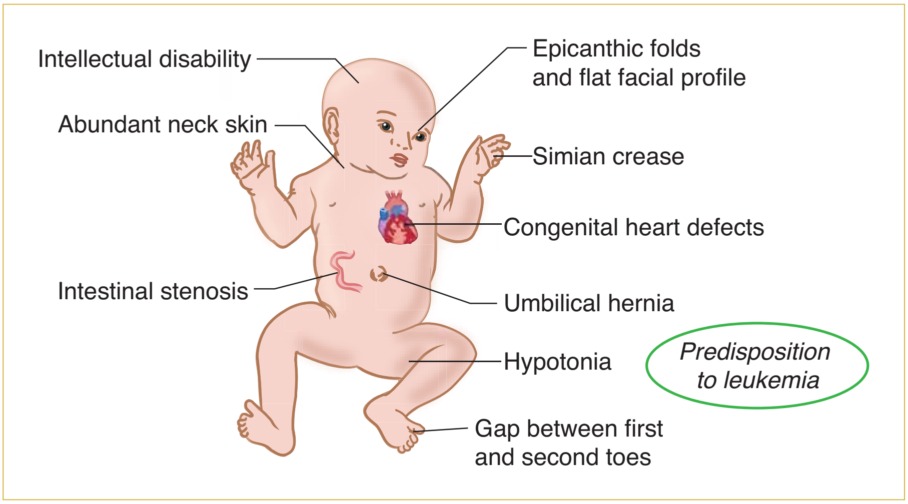

Figure 6-1. Down Syndrome

Edwards syndrome (trisomy 18) is caused by nondisjunction. The risk increases with maternal age.

Clinical findings can include intellectual disability; low-set ears and micrognathia; congenital heart defects; overlapping flexed fingers; and rocker-bottom feet. There is a very poor prognosis due to severe congenital malformations.

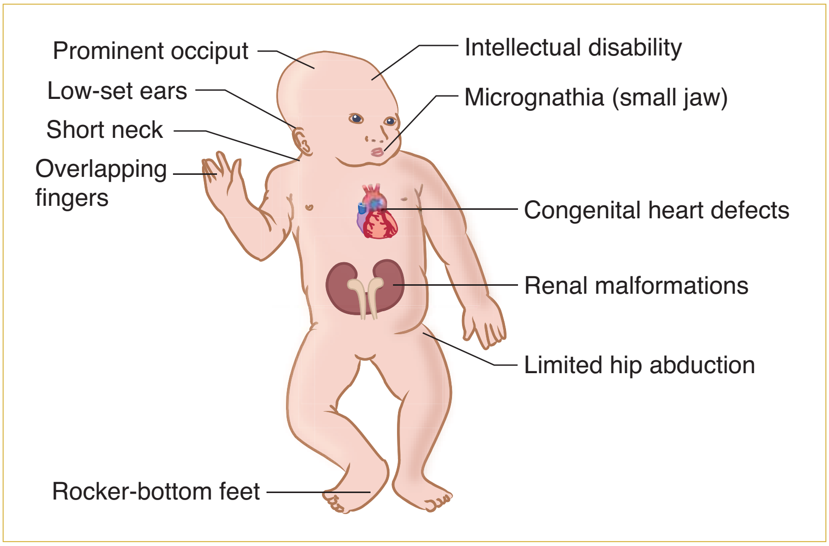

Figure 6-2. Edwards Syndrome

Patau syndrome (trisomy 13) is caused by nondisjunction. The risk increases with maternal age.

Clinical findings can include intellectual disability; cleft lip and/or palate; cardiac defects; renal abnormalities; microcephaly; holoprosencephaly; and polydactyly. The very poor prognosis is due to severe congenital malformations.

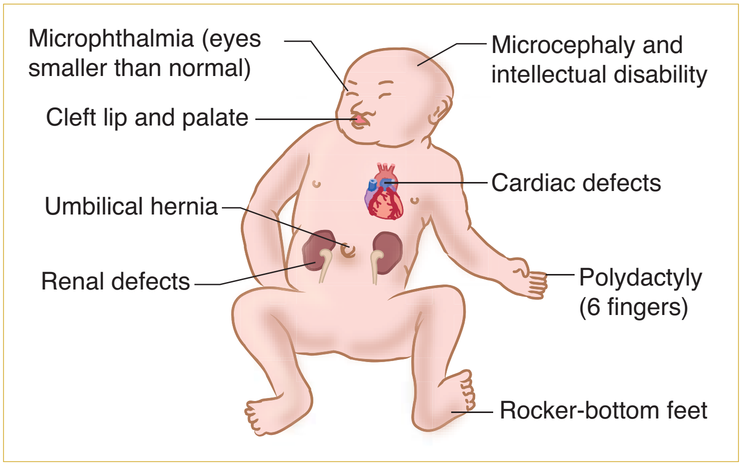

Figure 6-3. Patau Syndrome

DISORDERS INVOLVING CHROMOSOMAL DELETIONS

Cri du chat syndrome is due to deletion of the short arm of chromosome 5. Clinical findings include a characteristic high-pitched catlike cry; intellectual disability; congenital heart disease; and microcephaly. **Microdeletions** include 13q14 (retinoblastoma gene) and 11p13 (WAGR complex [Wilms tumor, aniridia, genitourinary anomalies, and intellectual disability [previously known as mental retardation]). Microdeletions are too small to be detected by karyotyping and require molecular techniques for detection.

DISORDERS INVOLVING SEX CHROMOSOMES

Klinefelter syndrome is caused by meiotic nondisjunction and is a common cause of male hypogonadism. The most common karyotype is 47,XXY. Lab studies show elevated FSH and LH with low levels of testosterone. Clinical findings include testicular atrophy, infertility due to azoospermia, eunuchoid body habitus, high-pitched voice; female distribution of hair; and gynecomastia.

Turner syndrome is a common cause of female hypogonadism. The most common karyotype is 45,X. The second X chromosome is necessary for oogenesis and normal development of the ovary. Clinically, patients fail to develop secondary sex characteristics and have short stature with widely spaced nipples. Other features include gonadal dysgenesis with atrophic streak ovaries; primary amenorrhea; and infertility.

Clinical features involving other organ systems include cystic hygroma and webbing of the neck; hypothyroidism; congenital heart disease (preductal coarctation of the aorta and bicuspid aortic valve); and hydrops fetalis. Females with 45,X/46,XY mosaicism are at risk for gonadoblastoma, microdeletions.

Note

The presence of a Y chromosome determines male phenotype due to the presence of the testes-determining factor gene (also called the sex-determining region Y [SRY]) on the Y chromosome.

Note

In **Lyon's hypothesis of X-inactivation**, only one X is genetically active. Most (though not all) of the genes on the other X chromosome are inactivated.

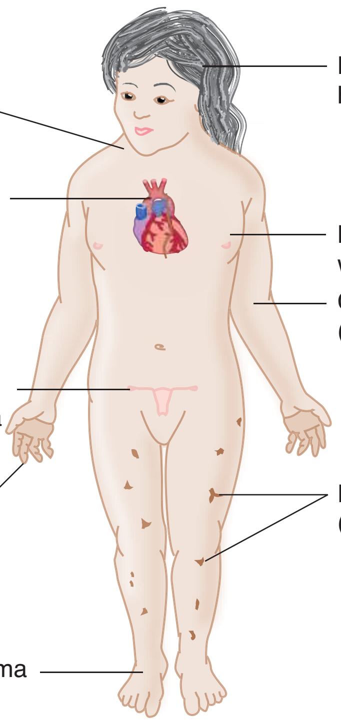

Figure 6-4. Turner Syndrome

DISORDERS OF SEXUAL DEVELOPMENT (DSD)

Determination of sex can be established by a variety of methods that do not necessarily completely agree.

- **Karyotypic** (genetic) sex refers to which sex chromosomes an individual has; the presence of a Y chromosome results in testicular development.
- **Gonadal** sex refers to the presence of ovarian or testicular tissue.
- **Ductal sex** refers to the presence of Müllerian (female: Fallopian tube, uterus, cervix, and upper portion of vagina) or Wolffian (male: epididymis, vas deferens, seminal vesicles, and ejaculatory ducts) duct adult derivatives.
- **Phenotypic** (genital) sex refers to the external appearance of the genitalia.

Individuals with **ovotesticular disorder** have both ovarian and testicular tissue, which is an extremely rare condition. The most common karyotype of ovotesticular disorder is 46,XX. The gonadal sex can be either an ovary on one side and testis on the other, or ovotestes, in which there is a gonad with both testicular and ovarian tissue. The ductal sex is often mixed, and the phenotypic sex shows ambiguous genitalia.

The **46,XX DSD category** includes individuals (formerly characterized as female pseudohermaphrodites) with disorders of ovarian development, androgen excess, vaginal atresia, and cloacal exstrophy. The **46,XY category** includes individuals (formerly characterized as male pseudohermaphrodites) with disorders of testicular development, disorders of androgen synthesis, severe hypospadias and cloacal exstrophy.

MENDELIAN DISORDERS

Mendelian disorders are characterized by single gene mutations. Common types of mutations include point mutations and frameshift mutations.

- **Point mutations** occur with a single nucleotide base substitution, which may produce a variety of effects. The form of point mutation called synonymous mutation (silent mutation) occurs when a base substitution results in a codon that codes for the same amino acid. The form of point mutation called missense mutation occurs when a base substitution results in a new codon and a change in amino acids. The form of point mutation called a nonsense mutation occurs when a base substitution produces a stop codon and therefore produces a truncated protein.
- **Frameshift mutations** occur when insertion or deletion of bases leads to a shift in the reading frame of the gene.

The location of a mutation will alter its potential effects. Mutations involving coding regions of DNA may result in abnormal amino acid sequences; decreased production of the protein; truncated or abnormally folded protein; or altered or lost function of the protein. Mutations of promoter or enhancer regions may interfere with transcription factors, resulting in decreased transcription of the gene.

Patterns of inheritance for genetic diseases show wide variation, and the genetic pattern of a disease may be classified as autosomal dominant; autosomal recessive; X-linked recessive; X-linked dominant; triplet repeat mutations; genomic imprinting; mitochondrial; or multifactorial.

Table 6-1. Autosomal Dominant and Recessive Diseases

	Autosomal Recessive	Autosomal Dominant
Onset	Early uniform onset (infancy/childhood)	Variable onset (may be delayed into adulthood)
Penetrance	Complete penetrance	Incomplete penetrance with variable expression
Mutation	Usually an enzyme protein	Usually a structural protein or receptor
Requires	Mutation of both alleles	Mutation of one allele

AUTOSOMAL RECESSIVE DISORDERS

(This material is included here for reinforcement. It is also covered in the Physiology Lecture Notes.)

Cystic fibrosis (CF) is the most common lethal genetic disorder in Caucasians. It is due to mutation of the chloride channel protein, cystic fibrosis transmembrane conductance regulator (CFTR), whose *CFTR* gene is located on chromosome 7 and most commonly has been damaged by a deletion of the amino acid phenylalanine at position 508 (ΔF508). The defective chloride channel protein leads to abnormally thick viscous mucus, which obstructs the ducts of exocrine organs.

Note

CF seems to break 2 rules of genetics:

- Inheritance is autosomal recessive, even though the affected protein is a receptor protein.
- The clinical manifestations vary widely, which is more characteristic of autosomal dominant disorders.

The **distribution of disease** reflects the distribution of eccrine sweat glands and exocrine glands.

- In the lungs, CF may cause recurrent pulmonary infections; chronic bronchitis; and bronchiectasis.
- In the pancreas, CF may cause plugging of pancreatic ducts resulting in atrophy and fibrosis; and pancreatic insufficiency leading to fat malabsorption, malodorous steatorrhea, and deficiency of fat-soluble vitamins.
- In the male reproductive system, CF may be associated with absence or obstruction of the vas deferens and epididymis, which often leads to male infertility.
- In the liver, plugging of the biliary canaliculi may result in biliary cirrhosis.
- In the GI tract, the thick secretions may cause small intestinal obstruction (meconium ileus).

Bridge to Biochemistry

Most CF cases result from deletion of phenylalanine at position 508 (ΔF508), which interferes with proper protein folding and the post-translational processing of oligosaccharide side chains. The abnormal chloride channel protein is degraded by the cytosolic proteasome complex rather than translocated to the cell membrane.

Diagnosis can be established with a sweat test (elevated NaCl) or DNA probes. Due to improved therapies, some patients live into their forties, but with this increase in longevity there has been an increase in liver disease. Patients succumb to pulmonary disease. The 3 most common pulmonary infections are *S. aureus, H. influenzae*, and *P. aeruginosa*. Lung transplantation is a treatment option. Patients infected with *Burkholderia cepacia* complex and who undergo transplant have a worse prognosis.

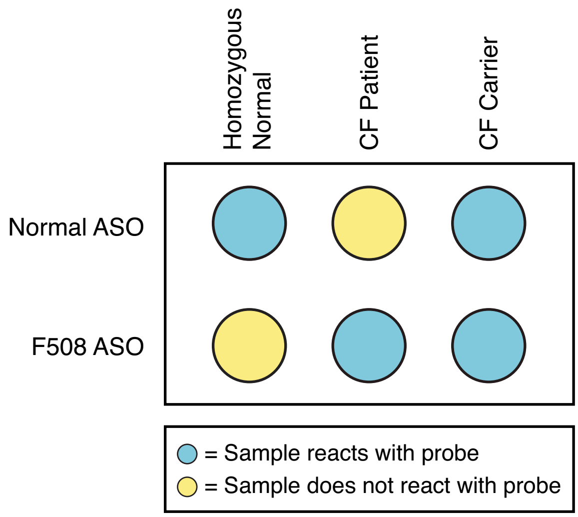

Figure 6-5. The Use of Allele-Specific Oligonucleotide (ASO) Probes for Cystic Fibrosis

Bridge to Biochemistry

Phenylalanine hydroxylase converts phenylalanine into tyrosine.

Phenylketonuria (PKU) is due to deficiency of phenylalanine hydroxylase, resulting in toxic levels of phenylalanine and a lack of tyrosine.

Clinically, affected children are normal at birth but, if undiagnosed and untreated, develop intellectual development disorder by age 6 months. The lack of tyrosine causes light-colored skin and hair, since melanin is a tyrosine derivative. Affected children may have a mousy or musty odor to the sweat and urine (secondary to metabolite [phenylacetate] accumulation).

Screening for PKU is done at birth. Treatment is dietary restriction of phenylalanine, including avoidance of the artificial sweetener aspartame.

A genetic variant, **benign hyperphenylalaninemia**, has partial enzyme deficiency with mildly increased levels of phenylalanine which are insufficient to cause intellectual disability.

In a minority of cases, an abnormality of the cofactor tetrahydrobiopterin causes a variant that does not respond to dietary restriction.

Transplacental accumulation of phenylalanine can cause problems with fetal development in cases of maternal PKU. Prevention requires maternal dietary restriction.

Alkaptonuria (ochronosis) occurs when deficiency of homogentisic acid oxidase results in the accumulation of homogentisic acid. The homogentisic acid has an affinity for connective tissues (especially cartilage), resulting in a black discoloration (as a consequence of oxidation of homogentisic acid).

Clinical features include urine that is initially pale yellow but turns black upon standing, and black-stained cartilage, which causes discoloration of the nose and ears. Alkaptonuria also predisposes for early onset of degenerative arthritis.

Albinism is caused by a lack of the enzyme tyrosinase needed for melanin production. Affected individuals show deficiency of melanin pigmentation in the skin, hair follicles, and eyes (oculocutaneous albinism), with resulting increased risk of basal cell and squamous cell carcinomas.

The **glycogen storage diseases** are a group of rare diseases that have in common a deficiency of one of the enzymes necessary for the metabolism of glycogen, which results in the accumulation of glycogen in the liver, heart, and skeletal muscle.

- **Type I (von Gierke disease)** is due to a deficiency of *glucose-6-phosphatase*, and is characterized clinically by hepatomegaly and hypoglycemia.
- **Type II (Pompe disease)** is due to a deficiency of *lysosomal α-1,4-glucosidase (acid maltase)*, and is characterized clinically by hepatomegaly, skeletal muscle hypotonia, cardiomegaly, and death from cardiac failure by age 2 years.
- **Type V (McArdle syndrome)** is due to a deficiency of *muscle glycogen phosphorylase*, and is characterized clinically by exercise-induced muscle cramps.

Tay-Sachs disease is due to a deficiency of hexosaminidase A (due to mutation of *HEXA* gene on chromosome 15), which leads to the accumulation of GM2 ganglioside in the lysosomes of the CNS and retina. Tay-Sachs is common in Ashkenazi Jews (1 in 30 carrier rate).

The distribution of disease involves the retina (cherry-red spot due to accentuation of the macula) and central nervous system (dilated neurons with cytoplasmic vacuoles). Affected children are normal at birth, but by 6 months show onset of symptoms (progressive mental deterioration and motor incoordination) that progress to death by age 2–3 years. **Electron microscopy** shows distended lysosomes with whorled membranes; the diagnosis can also be established with enzyme assays and DNA probes.

Note

Lysosomal Storage Diseases

Defined as a deficiency of a lysosomal enzyme (acid hydrolase), which leads to the accumulation of a complex substrate within the lysosome leading to enlarged cells that become dysfunctional

- Tay-Sachs
- Niemann-Pick
- Gaucher
- Mucopolysaccharidosis
- Fabry
- Metachromatic leukodystrophy

Table 6-2. Lysosomal Storage Diseases

Disease	Enzyme Deficiency	Accumulating Substance
Tay-Sachs disease	Hexosaminidase A	GM_2 ganglioside
Niemann-Pick disease types A and B	Sphingomyelinase	Sphingomyelin
Gaucher disease	Glucocerebrosidase	Glucocerebroside
Fabry disease	α -galactosidase A	Ceramide trihexoside
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide
Hurler syndrome	α -L-iduronidase	Dermatan sulfate Heparan sulfate
Hunter syndrome	L-iduronate sulfatase	Dermatan sulfate Heparan sulfate

Note

"Zebra bodies" are concentric lamellated inclusions seen in the cytoplasm on electron microscopy. Although they are present in Niemann-Pick disease, they are also present in other diseases including Fabry disease and Hurler syndrome.

Niemann-Pick disease is caused by a deficiency of sphingomyelinase, which leads to the accumulation of sphingomyelin within the lysosomes of the CNS and reticuloendothelial system (monocytes and macrophages located in reticular connective tissue). Niemann-Pick is common in Ashkenazi Jews (note similarity to Tay-Sachs disease).

The distribution of disease depends on the form of disease, but can involve the retina (cherry-red spot, note similarity to Tay-Sachs disease); central nervous system (distended neurons with a foamy cytoplasmic vacuolization, note similarity to Tay-Sachs disease); and reticuloendothelial system (hepatosplenomegaly, lymphadenopathy, and bone marrow involvement; note difference from Tay-Sachs disease).

In Niemann-Pick **types A and B**, there is a mutation affecting an enzyme that metabolizes lipids; organomegaly occurs, and with type A, there is severe neurologic damage. In **type C**—the most common form—a defect in cholesterol transport causes ataxia, dysarthria, and learning difficulties. All forms are lethal, usually before adulthood.

Gaucher disease is the most common lysosomal storage disorder. Deficiency of glucocerebrosidase leads to the accumulation of glucocerebroside, predominately in the lysosomes of the reticuloendothelial system (monocytes and macrophages located in reticular connective tissue).

Type I represents 99% of cases and presents in adulthood with hepatosplenomegaly; thrombocytopenia/pancytopenia secondary to hypersplenism; lymphadenopathy; and bone marrow involvement that may lead to bone pain, deformities, and fractures. Central nervous system manifestations occur in **types II and III**.

The characteristic **Gaucher cells** are enlarged macrophages with a fibrillary (tissue paper–like) cytoplasm. Diagnosis can be established with biochemical enzyme assay of glucocerebrosidase activity.

Mucopolysaccharidosis (MPS) is a group of lysosomal storage disorders characterized by deficiencies in the lysosomal enzymes required for the degradation of mucopolysaccharides (glycosaminoglycans).

Clinical features include intellectual disability; cloudy cornea; hepatosplenomegaly; skeletal deformities and coarse facial features; joint abnormalities; and cardiac lesions. MPS I (Hurler syndrome) is the severe form and is due to deficiency of α-L-iduronidase. MPS II (Hunter syndrome) is a milder form; it shows X-linked recessive inheritance and is due to a deficiency of L-iduronate sulfatase.

AUTOSOMAL DOMINANT DISORDERS

Familial hypercholesterolemia is the most common inherited disorder (incidence 1 in 500) and is due to a mutation in the low density lipoprotein (LDL) receptor gene (LDLR) on chromosome 19. The mutations in the LDL receptor cause increased levels of circulating cholesterol, loss of feedback inhibition of HMG-coenzyme A (HMG-CoA) reductase, and increased phagocytosis of LDL by macrophages.

There are 5 major **classes of mutation**.

- **Class I:** no LDL receptor synthesis
- **Class II:** defect in transport out of the endoplasmic reticulum
- **Class III:** defect in LDL receptor binding
- **Class IV:** defect in ability to internalize bound LDL
- **Class V:** defect in the recycling of the LDL receptor

Bridge to Biochemistry

HMG-CoA reductase is the rate-limiting enzyme in the synthesis of cholesterol. Normally, cholesterol represses the expression of the HMG-CoA reductase gene (negative feedback).

Clinical features include elevated serum cholesterol (heterozygotes have elevations of 2–3 times the normal level and homozygotes have elevations of 5–6 times the normal level), skin xanthomas (collections of lipid-laden macrophages), xanthelasma around the eyes, and premature atherosclerosis (homozygotes often develop myocardial infarctions in late teens and twenties).

Marfan syndrome is due to a mutation of the **fibrillin** gene (*FBN1*) on chromosome 15q21. Fibrillin is a glycoprotein that functions as a scaffold for the alignment of elastic fibers.

Clinical features include skeletal changes (tall, thin build with long extremities, hyperextensible joints, pectus excavatum [inwardly depressed sternum], and pectus carinatum [pigeon breast]) and abnormal eyes (ectopia lentis, characterized by bilateral subluxation of the lens). The cardiovascular system is also particularly vulnerable; it may show cystic medial degeneration of the media of elastic arteries with a loss of elastic fibers and smooth muscle cells with increased risk of dissecting aortic aneurysm (a major cause of death), dilatation of the aortic ring potentially leading to aortic valve insufficiency, and/or mitral valve prolapse.

Ehlers-Danlos syndrome (EDS) is a group of inherited connective tissue diseases that have in common a defect in collagen structure or synthesis. Clinically, the disease causes hyperextensible skin that is easily traumatized and hyperextensible joints secondary to effects on the joints and adjacent ligaments.

There are a number of variants with different modes of inheritance.

- **Kyphoscoliotic EDS**: autosomal recessive form
- **Vascular variant EDS**: autosomal dominant form that causes rupture of vessels and bowel wall
- **Classical EDS**: autosomal dominant form that causes a type V collagen defect; patients have a normal lifespan

Note

Disorders of collagen biosynthesis include scurvy, osteogenesis imperfecta, Ehlers-Danlos syndrome, Alport syndrome, and Menkes disease.

Neurofibromatosis

Type 1 (**von Recklinghausen disease**) neurofibromatosis (90% of cases) has an incidence of 1 in 3,000. The condition is due to a mutation of the tumor suppressor gene *NF1* located on chromosome 17 (17q11.2). The normal gene product (neurofibromin) inhibits p21 ras oncoprotein.

- Affected individuals characteristically have multiple neurofibromas and benign tumors of peripheral nerves that are often numerous and may be disfiguring. The plexiform variant of the neurofibromas is diagnostic.
- Rarely (3%), malignant transformation of a neurofibroma may occur.
- Other clinical features include pigmented skin lesions (6 or more "cafe-au-lait spots" that are light brown macules usually located over nerves); pigmented iris hamartomas (Lisch nodules); and increased risk of meningiomas and pheochromocytoma, an adrenal tumor that also occurs with von Hippel-Lindau disease and MEN 2.

Type 2 (**bilateral acoustic**) neurofibromatosis (10% of cases) has an incidence of 1 in 45,000.

- There is a mutated tumor suppressor gene *NF-2* (22q12.2) on chromosome 22.
- The normal gene product (merlin) is a critical regulator of contact-dependent inhibition of proliferation.
- Clinical features include vestibular schwannomas (acoustic neuromas), and increased risk of meningioma and ependymomas.

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Figure 6-6. Multiple Subcutaneous Neural Tumors of Neurofibromatosis

von Hippel-Lindau disease is due to a mutation of the tumor suppressor gene *VHL* on chromosome 3p (3p26-p25). The normal gene product's main action is to tag proteins (e.g., hypoxia inducible factor 1a [a transcription factor that induces the expression of angiogenesis factors]) with ubiquitin for degradation.

Clinical manifestations can include retinal hemangioblastoma (von Hippel tumor); hemangioblastomas of the cerebellum, brain stem, and spinal cord (Lindau tumor); cysts of the liver, pancreas, and kidneys; and multiple bilateral renal cell carcinomas.

X-LINKED RECESSIVE CONDITIONS

In **X-linked recessive conditions**, males with a mutant recessive gene on the X chromosome have the condition, while daughters of affected males are obligate carriers, who in many situations are asymptomatic.

- Sons of affected males do not carry the mutation.
- Daughters of carrier females may be either normal or carriers.
- Sons of carrier females may be affected or normal (because males are hemizygous for the X chromosome).

Lesch-Nyhan syndrome results from deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT), which impairs salvaging of the purines hypoxanthine and guanine. Clinical features include intellectual disability, hyperuricemia, and self-mutilation.

Testicular feminization is an androgen insensitivity that causes failure of normal masculinization of external genitalia of XY males.

In **Bruton agammaglobulinemia**, defective Bruton tyrosine kinase (*Btk*) at band Xq21.3 causes complete failure of immunoglobulin production characterized clinically by complete absence of antibodies in serum and recurrent bacterial infections. (*See* the Immunopathology chapter 7.)

In **Menkes disease**, a mutation of the *ATP7A* gene impairs copper distribution. Infants show failure to thrive, and death occurs in the first decade.

Note

Most common genetic causes of intellectual developmental disorder:

- Down syndrome
- Fragile X syndrome

X-LINKED DOMINANT CONDITIONS

X-linked dominant conditions are similar to X-linked recessive, but both males and females show disease. An example is Alport syndrome, which is a hereditary glomerulonephritis with nerve deafness. Alport syndrome can also be inherited in other patterns.

Note

Triplet repeat expansion can occur in a coding region (Huntington and spinobulbar muscular atrophy) or in an untranslated region of the gene (fragile X and myotonic dystrophy).

TRIPLET REPEAT MUTATIONS

Fragile X syndrome is due to triplet nucleotide repeat mutations, so that the nucleotide sequence CGG repeats typically hundreds to thousands of times. The mutation occurs in the *FMR-1* gene (fragile X mental retardation-1) on the X chromosome (Xq27.3), and the disease behaves as an X-linked dominant disease that causes intellectual disability in all affected males and 50% of female carriers. The characteristic phenotype includes elongated face with a large jaw, large everted ears, and macroorchidism. The condition can be diagnosed with DNA probe analysis.

Huntington disease is due to a triplet repeat mutation (CAG) of the *HTT* gene that produces an abnormal protein (huntingtin), which is neurotoxic and causes atrophy of the caudate nucleus. Huntington disease has an early onset (age range: 20–50 years) of progressive dementia with choreiform movements.

GENOMIC IMPRINTING

Genomic imprinting refers to differential expression of genes based on chromosomal inheritance from maternal versus paternal origin.

- In **Prader-Willi syndrome**, microdeletion on paternal chromosome 15 {del(15)(q11;q13)} causes intellectual disability, obesity, hypogonadism, and hypotonia.
- In **Angelman syndrome**, microdeletion on maternal chromosome 15 {del(15)(q11;q13)} causes intellectual disability, seizures, ataxia, and inappropriate laughter.

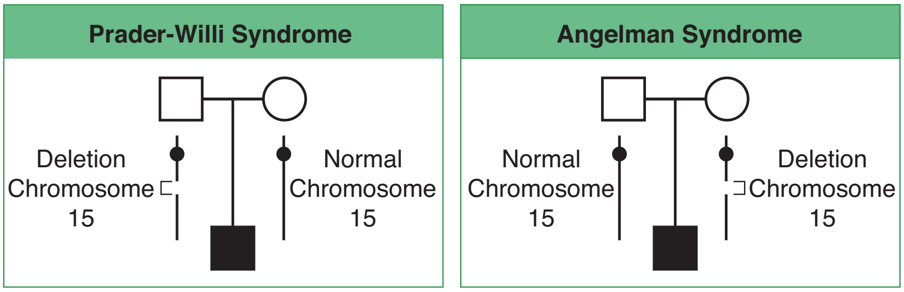


Figure 6-7. Genomic Imprinting

The inheritance of a deletion on chromosome 15 from a male produces Prader-Willi syndrome, whereas inheritance of the same deletion from a female produces Angelman syndrome.

MITOCHONDRIAL DNA DISORDERS

Mitochondrial DNA codes for mitochondrial oxidative phosphorylation enzymes; inheritance is only from mother to child, because only the ovum contributes mitochondria to the zygote. Examples include:

- **Leber hereditary optic neuropathy** causes loss of retinal cells, which leads to central vision loss.
- **Myoclonic epilepsy with ragged red fibers (MERRF)** is a mitochondrial disorder characterized by epilepsy, ataxia, peripheral neuropathy and deterioration in cognitive ability. Sensorineural hearing loss and ocular dysfunction can also develop. Patients have short stature and cardiomyopathy. On muscle biopsy, ragged red fibers are seen on Gomori trichrome staining due to the accumulation of mitochondria.

MULTIFACTORIAL INHERITANCE

Multifactorial inheritance refers to disease caused by a combination of multiple minor gene mutations and environmental factors. Examples include open neural tube defects and type 2 diabetes mellitus.

Immunopathology 7

Learning Objectives

- ❑ Explain information related to hypersensitivity reactions and autoimmune diseases
- ❑ Answer questions about primary/secondary immune deficiency syndromes
- ❑ Demonstrate understanding of AIDS
- ❑ Answer questions about immunology of transplant rejection

HYPERSENSITIVITY REACTIONS

(This material is included here for reinforcement. It is also covered in the Immunology Lecture Notes.)

Type I (immediate) hypersensitivity reactions **(anaphylactic type)** are characterized by IgE-dependent release of chemical mediators from mast cells and basophils. Cross-linking of IgE bound to antigen to IgE Fc receptors on the surface of mast cells and basophils causes degranulation. This binding triggers release of chemical mediators that include histamine and heparin; eosinophil chemotactic factor; leukotriene B4 and neutrophil chemotactic factor; and prostaglandin D4, platelet-activating factor (PAF), and leukotrienes C4 and D4. Influx of eosinophils amplifies and perpetuates the reaction. Effects may be systemic (anaphylaxis, as for example due to bee stings or drugs) or localized (food allergies, atopy, and asthma).

Type II hypersensitivity reactions (antibody-mediated) are mediated by IgG or IgM antibodies directed against a specific target cell or tissue. Reactions can take several forms.

- In complement-dependent cytotoxicity, fixation of complement results in osmotic lysis or opsonization of antibody-coated cells; examples include autoimmune hemolytic anemia, transfusion reactions, and erythroblastosis fetalis.
- In antibody-dependent cell-mediated cytotoxicity (ADCC), cytotoxic killing of an antibody-coated cell occurs; an example is pernicious anemia. Antireceptor antibodies can activate or interfere with receptors; examples include Graves disease and myasthenia gravis.

Type III hypersensitivity reactions **(immune complex disease)** are characterized by the formation of in situ or circulating antibody-antigen immune complexes, which deposit in tissue resulting in inflammation and tissue injury. Examples include serum sickness, systemic lupus erythematosus (SLE), and glomerulonephritis.

Type IV hypersensitivity reactions **(cell-mediated type)** are mediated by sensitized T lymphocytes. In delayed type hypersensitivity, CD4+ TH1 lymphocytes mediate granuloma formation; examples include the PPD skin test and tuberculosis.

In cytotoxic T-cell–mediated hypersensitivity, CD8+ T-cell lymphocytes destroy antigen-containing cells; examples include type 1 diabetes, virus-infected cells, immune reaction to tumor-associated antigens, and graft rejection.

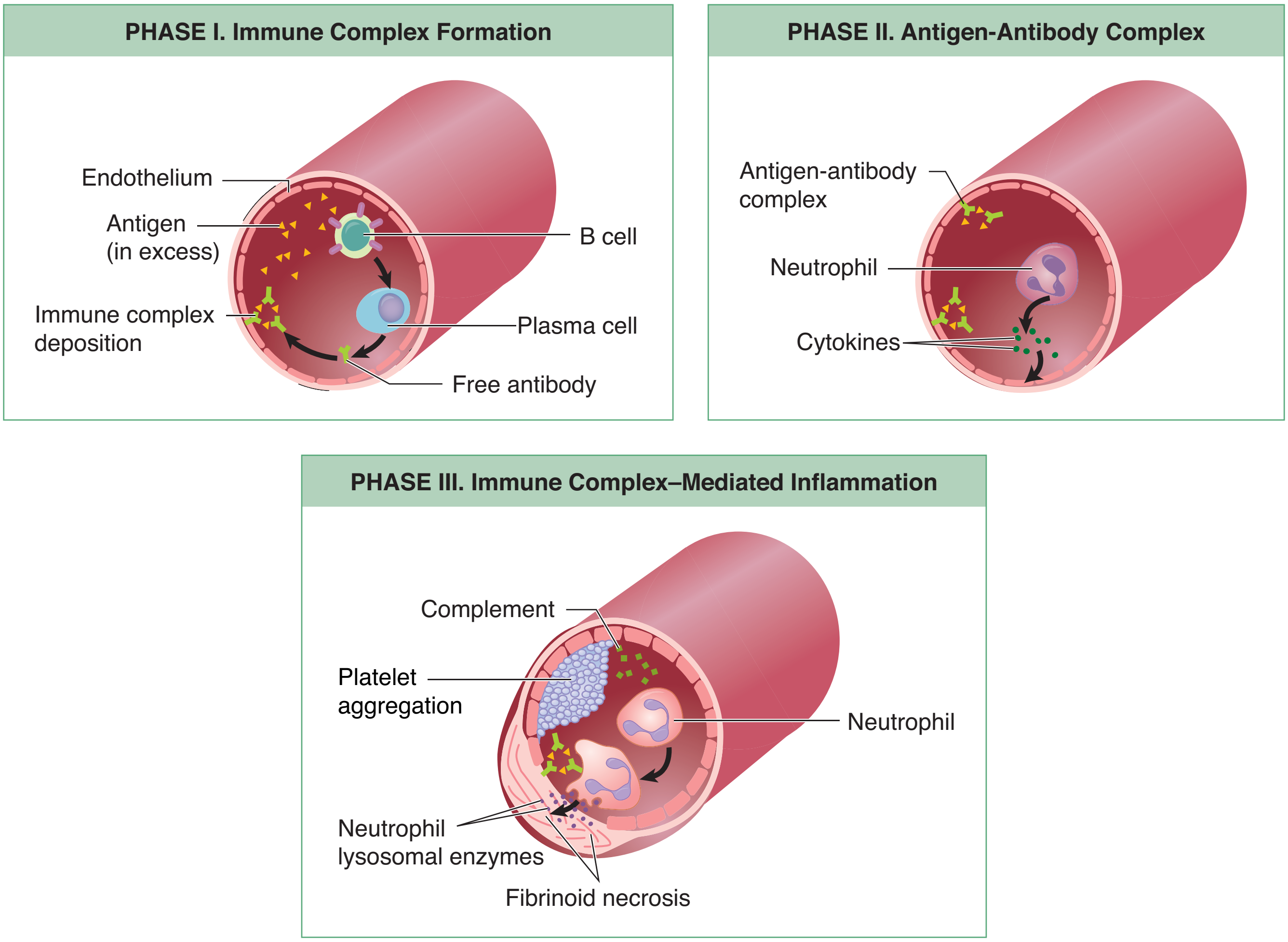


Figure 7-1. Type III Hypersensitivity

Note

Multiple autoantibodies may be produced and are commonly directed against nuclear antigens (DNA, histones, nonhistone nuclear RNA proteins) and blood cells.

AUTOIMMUNE DISEASES

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by loss of self-tolerance and production of autoantibodies. Females are affected much more often than males (M:F = 1:9); peak incidence is age 20–45; and African Americans are affected more often than Caucasians. The mechanism of injury in lupus is a mix of type II and III hypersensitivity reactions.

- Important **autoantibodies** that may be detected in the sera from lupus patients include antinuclear antibody (ANA) (>95%); anti-dsDNA (40–60%); anti-Sm (20–30%); antihistone antibodies; nonhistone nuclear RNA proteins; and blood cells.

- SLE affects **many organ systems**.
  - Hematologic (type II hypersensitivity reaction) manifestations can include hemolytic anemia, thrombocytopenia, neutropenia, and lymphopenia.
  - Skeletal manifestations include arthritis characterized by polyarthralgia and synovitis without joint deformity (type III hypersensitivity reaction).
  - Skin (type III hypersensitivity reaction) manifestations can include a malar "butterfly" rash; maculopapular rash; and ulcerations and bullae formation.
  - Serosal surfaces may also be affected, with resulting pericarditis, pleuritis, or pleural effusions (type III hypersensitivity reaction).
  - Central nervous system manifestations include focal neurologic symptoms, seizures, and psychosis (type III hypersensitivity reaction).
  - Cardiac manifestations include Libman-Sacks endocarditis (nonbacterial verrucous endocarditis) (type III hypersensitivity reaction).
- Of particular importance are the renal manifestations (type III hypersensitivity) classified by the Society of Nephrology/Renal Pathology Society as follows.
  - **Class I**: minimal mesangial lupus nephritis
  - **Class II**: mesangial proliferative lupus nephritis
  - **Class III**: focal (< 50%) lupus nephritis
  - **Class IV**: diffuse (> 50%) lupus nephritis
  - **Class V**: membranous lupus nephritis
  - **Class VI**: advanced sclerosing lupus nephritis
- Lupus is treated with **steroids** and **immunosuppressive agents**. It tends to have a chronic, unpredictable course with remissions and relapses. The 10-year survival is 85%, with death frequently being due to renal failure or infections.

Sjögren syndrome (sicca syndrome) is an autoimmune disease characterized by destruction of the lacrimal and salivary glands, resulting in the inability to produce saliva and tears. Females are affected more often than males, with typical age 30–50.

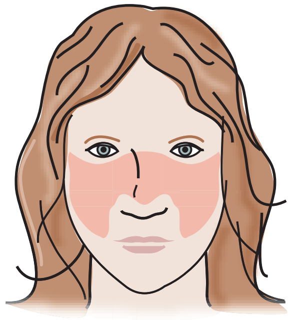
Clinical manifestations include keratoconjunctivitis sicca (dry eyes) and corneal ulcers; xerostomia (dry mouth); and Mikulicz syndrome (enlargement of the salivary and lacrimal glands). Sjögren syndrome is often associated with rheumatoid arthritis and other autoimmune diseases. The characteristic autoantibodies are the anti-ribonucleoprotein antibodies SS-A (Ro) and SS-B (La). There is an increased risk of developing non-Hodgkin lymphoma.

Scleroderma (progressive systemic sclerosis) is an autoimmune disease characterized by fibroblast stimulation and deposition of collagen in the skin and internal organs. It affects females more than males, with typical age range of 20 to 55 years. The pathogenesis involves activation of fibroblasts by cytokines interleukin 1 (IL-1), platelet-derived growth factor (PDGF), and/or fibroblast growth factor (FGF) with the resulting activated fibroblasts causing fibrosis.

- **Diffuse scleroderma** has anti-DNA topoisomerase I antibodies (Scl-70) (70%), widespread skin involvement, and early involvement of the visceral organs. Organs that can be affected include the esophagus (dysphagia), GI tract (malabsorption), lungs (pulmonary fibrosis which causes dyspnea on

Clinical Correlate

Antihistone antibodies: Hydralazine, isoniazid, and procainamide can cause a lupus-like syndrome with antihistone antibodies.



Butterfly Rash

Clinical Correlate

Maternal **anti-Ro (SS-A) antibodies** have been implicated in the pathogenesis of congenital complete heart block.

Note

CREST Syndrome

- Calcinosis
- Raynaud phenomenon
- Esophageal dysmotility
- Sclerodactyly
- Telangiectasia

exertion), heart (cardiac fibrosis which may manifest as arrhythmias), and kidney (fibrosis that may manifest as renal insufficiency).

- **Localized scleroderma** (CREST syndrome) has anti-centromere antibodies, skin involvement of the face and hands, late involvement of visceral organs, and a relatively benign clinical course.

Dermatomyositis and polymyositis. See Skeletal Muscle chapter.

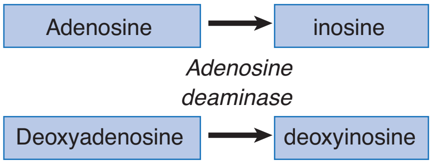
Mixed connective tissue disease is an overlap condition with features of systemic lupus erythematosus, systemic sclerosis, and polymyositis. Antiribonucleoprotein antibodies are nearly always positive.

PRIMARY IMMUNE DEFICIENCY SYNDROMES

X-linked agammaglobulinemia of Bruton is an immunodeficiency characterized by a developmental failure to produce mature B cells and plasma cells, resulting in agammaglobulinemia. The condition occurs because of loss of function mutations of B-cell Bruton tyrosine kinase (BTK). Clinically, the disease affects male infants who have recurrent infections beginning at 6 months of life due to the loss of passive maternal immunity. Common infections include pharyngitis, otitis media, bronchitis, and pneumonia; common infecting organisms include *H. influenza, S. pneumococcus*, and *S. aureus.*

Common variable immunodeficiency is a group of disorders characterized by defects in B-cell maturation that can lead to defective IgA or IgG production. Clinically, both sexes are affected with onset in childhood of recurrent bacterial infections and with increased susceptibility to *Giardia lamblia*. Complications include increased frequency of developing autoimmune disease, non-Hodgkin lymphoma, and gastric cancer.

Note



Adenosine deaminase is an important enzyme in purine metabolism; a deficiency of it results in accumulation of deoxyadenosine within lymphoid progenitor cells.

DiGeorge syndrome is an embryologic failure to develop the 3rd and 4th pharyngeal pouches, resulting in the absence of the parathyroid glands and thymus. Clinical findings can include neonatal hypocalcemia and tetany, T-cell deficiency, and recurrent infections with viral and fungal organisms.

Severe combined immunodeficiency (SCID) is a combined deficiency of cell-mediated and humoral immunity that is often caused by a progenitor-cell defect. The modes of inheritance are variable and can include X-linked (mutation of the common [gamma] chain of the interleukin receptors IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) and autosomal recessive (deficiency of adenosine deaminase). Clinical features include recurrent infections with bacteria, fungi, viruses, and protozoa; susceptibility to *Candida*, cytomegalovirus (CMV) and *Pneumocystis jiroveci* infections, and adverse reactions to live virus immunizations. SCID is treated with hematopoietic stem cell transplantation since the prognosis without treatment is death of most infants within a year.

Wiskott-Aldrich syndrome is an X-linked recessive disease with mutation in the gene for Wiskott-Aldrich syndrome protein (WASP). The disease has a clinical triad of recurrent infections, severe thrombocytopenia, and eczema (chronic spongiform dermatitis). Treatment is hematopoietic stem cell transplantation. Complications include increased risk of non-Hodgkin lymphoma and death due to infection or hemorrhage.

Complement system disorders can involve a variety of factors, with deficiencies of different factors producing different clinical patterns.

In both the classical and alternate pathways, C3 deficiency causes both recurrent bacterial infections and immune complex disease, while C5, C6, C7, and C8 deficiencies cause recurrent meningococcal and gonococcal infections.

- In the classical pathway only, C1q, C1r, C1s, C2, and C4 deficiencies cause marked increases in immune complex diseases, including infections with pyogenic bacteria.
- In the alternate pathway, Factor B and properdin deficiencies cause increased neisserial infections. Deficiencies in complement regulatory proteins can cause C1-INH deficiency (hereditary angioedema), which is characterized clinically by edema at mucosal surfaces with low C2 and C4 levels.

MHC class II deficiency can be caused by defects in positive selection of thymocytes. Few CD4+ lymphocytes develop and as a result, patients suffer from severe immunodeficiency. Mutations in genes (i.e., *CIITA*) that encode proteins that regulate MHC class II gene expression are the cause. CD8+ T cells are unaffected.

Hyper IgM syndrome is characterized by normal B and T lymphocyte numbers and normal to elevated IgM levels but significantly decreased IgA, IgG and IgE levels. Mutations in the gene for CD40 ligand result in the most common form of X-linked hyper IgM syndrome.

Selective IgA deficiency has unknown genetic etiology. Many affected individuals appear healthy while others have significant illness. Sinopulmonary infections, diarrhea and adverse reactions to transfusions can occur. Levels of IgA are undetectable whereas levels of other isotypes are normal. There is an association with autoimmune disease.

Phagocyte deficiencies (*See* chronic granulomatous disease in Inflammation chapter.)

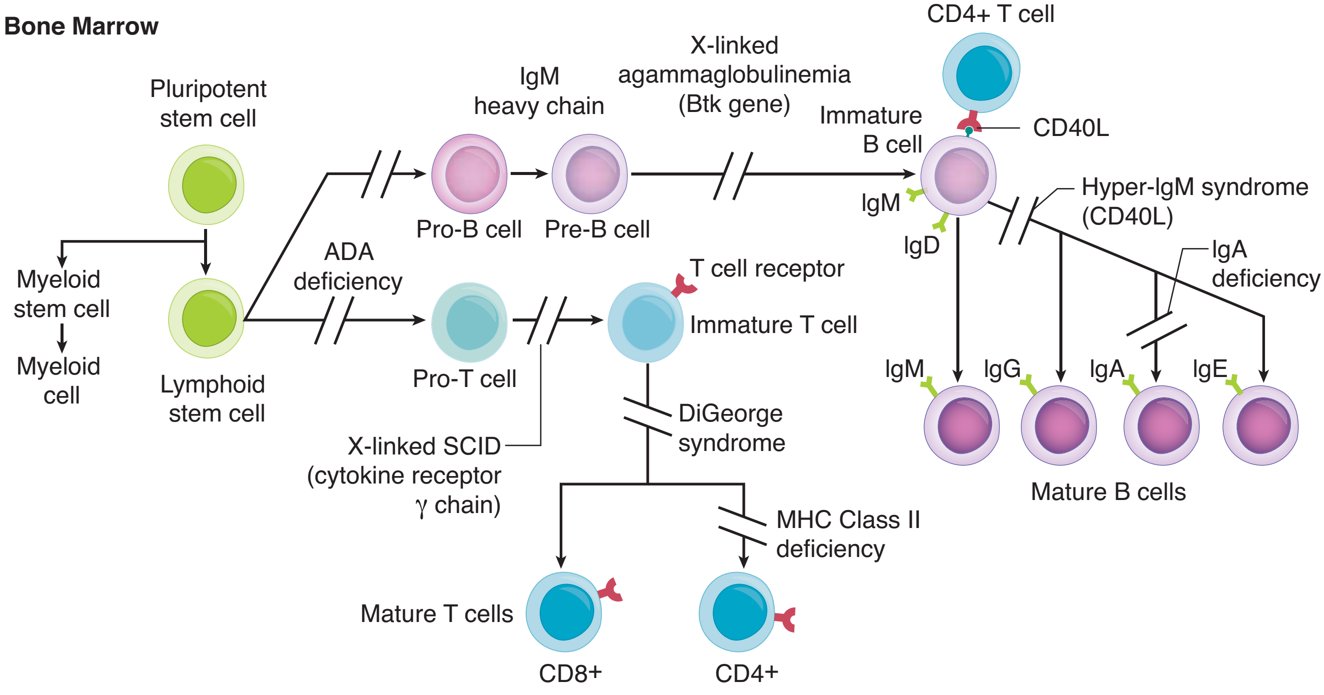


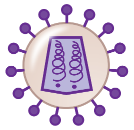
Figure 7-2. Primary Immune Deficiency Syndromes

SECONDARY IMMUNE DEFICIENCY SYNDROMES

Systemic diseases that can cause secondary immunodeficiency include diabetes mellitus, collagen vascular disease (e.g., systemic lupus erythematosus), and chronic alcoholism. Secondary immunodeficiency is more common.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

AIDS can be diagnosed when a person is HIV-positive and has CD4 count <200 cells/mL, **or** when a person is HIV-positive and has an AIDS-defining disease. Males are affected more frequently than females.



The ***human immunodeficiency virus*** (HIV) is an enveloped RNA retrovirus that contains reverse transcriptase. HIV infects CD4-positive cells, including CD4+ T lymphocytes, all macrophages, lymph node follicular dendritic cells, and Langerhans cells. The mechanism of infection is by binding of CD4 by the viral gp120, followed by entry into cell by fusion, which requires gp41 and coreceptors CCR5 (β-chemokine receptor 5) and CXCR4 (α-chemokine receptor).

Transmission of HIV can occur by many mechanisms, including sexual contact (most common mode, including both homosexual transmission and an increasing rate of heterosexual transmission, with important cofactors including herpes and syphilis infection); parenteral transmission; IV drug use; blood transfusions (including those done in hemophiliacs); accidental needle sticks in hospital workers; and vertical transmission.

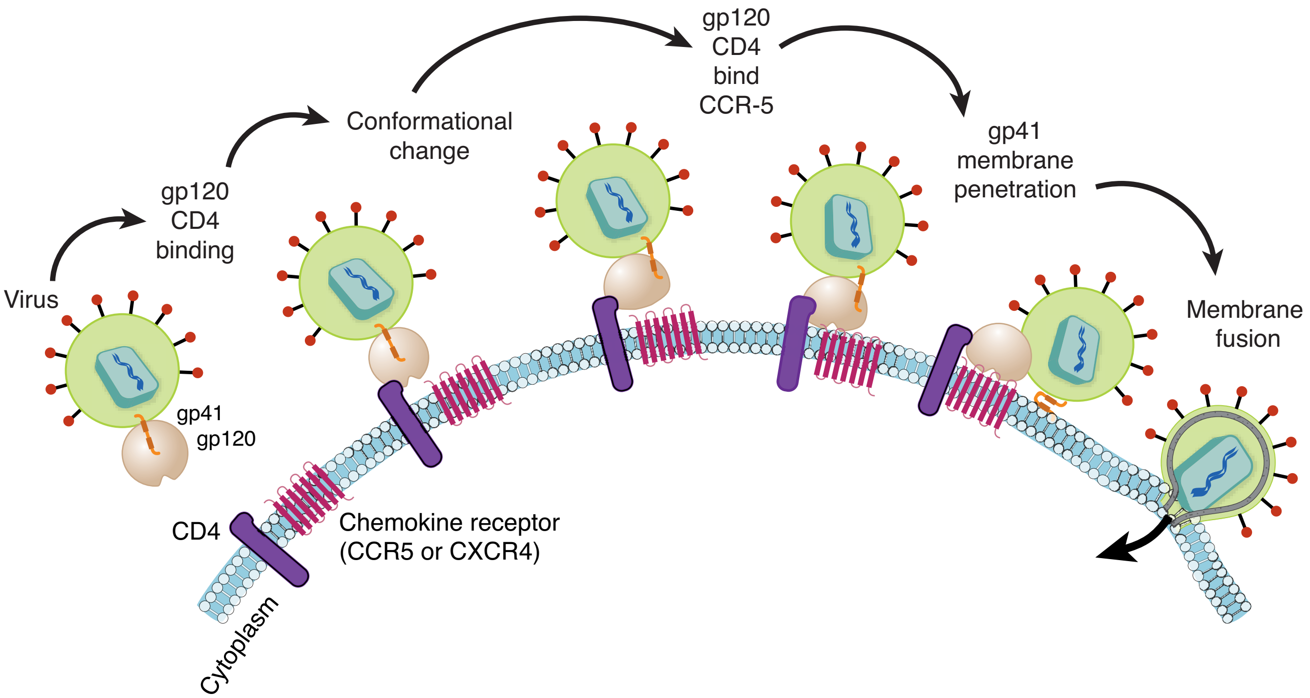


Figure 7-3. Mechanisms of HIV Infection

Diagnosis. The CDC recommends initial testing with an antigen/antibody combination immunoassay, followed by a confirmatory HIV-1/HIV-2 antibody differentiation immunoassay. If the confirmatory test is negative, testing with an HIV-1 nucleic acid test is done. Treatment varies, and can include combination antiretroviral treatment, reverse transcriptase inhibitors, protease inhibitors, and prophylaxis for opportunistic infections based on CD4 count.

The clinical manifestations of HIV infection vary over time.

- The **acute phase** is characterized by viremia with a reduction in CD4 count, mononucleosis-like viral symptoms and lymphadenopathy, and seroconversion.
- The **latent phase** is characterized by asymptomatic or persistent generalized lymphadenopathy with continued viral replication in the lymph nodes and spleen, low level of virus in the blood, and minor opportunistic infections including oral thrush (candidiasis) and herpes zoster. The average duration of latent phase is 10 years.
- **Progression to AIDS** (third phase) occurs with reduction of CD4 count to <200 cells/mL, which is accompanied by reemergence of viremia and development of AIDS-defining diseases, possibly to eventual death.

Table 7-1.
Opportunistic Infection and Common Sites of Infection in AIDS Patients

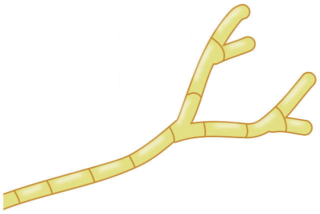
Opportunistic Infection	Common Sites of Infection
Pneumocystis jiroveci	Lung (pneumonia), bone marrow
Mycobacterium tuberculosis	Lung, disseminated
Mycobacterium avium-intracellulare	Lung, GI tract, disseminated
Coccidioidomycosis	Lung, disseminated
Histoplasmosis	Lung, disseminated
Cytomegalovirus	Lung, retina, adrenals, and GI tract
Giardia lamblia	GI tract
Cryptosporidium	GI tract
Herpes simplex virus	Esophagus and CNS (encephalitis)
Candida	Oral pharynx and esophagus
Aspergillus	CNS, lungs, blood vessels
Toxoplasmosis	CNS
Cryptococcus	CNS (meningitis)
JC virus	CNS (progressive multifocal leukoencephalopathy)
Bartonella spp.	Skin, mucosa, bone (bacillary angiomatosis)

Note

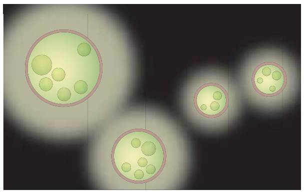
Macrophages and follicular dendritic cells are reservoirs for the virus.

Note

The CD4+ cell count is used to determine the health of the immune system and for recommendations on instituting prophylaxis for opportunistic diseases. Viral load is followed to assess treatment efficacy.



Septate Hyphae
(*Aspergillus*)



Cryptococcus neoformans

AIDS-Defining Diseases

Hairy leukoplakia is an Epstein-Barr virus (EBV)–associated condition due to infection of squamous cells. White plaques are present on the tongue.

Kaposi sarcoma is the most common neoplasm in AIDS patients. (*See* Vascular Pathology chapter.)

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Figure 7-4. Kaposi Sarcoma in an AIDS Patient

Non-Hodgkin lymphomas tend to be high-grade B-cell lymphomas; extranodal CNS lymphomas are common.

Other AIDS-defining diseases include cervical cancer, HIV-wasting syndrome, AIDS nephropathy, and AIDS dementia complex.

IMMUNOLOGY OF TRANSPLANT REJECTION

Rejection is caused largely by differences in HLA alleles between donor and recipient. Immunosuppressive agents are used to prevent and mitigate rejection.

- **Hyperacute rejection** occurs within minutes to hours due to preformed antibodies in the recipient. Lymphocyte cross-matching has almost eliminated this problem.
- **Acute rejection** occurs in the first 6 months and may be cellular (CD8+ T lymphocytes kill graft cells) or antibody-mediated.
- **Chronic rejection** occurs after months or years and may be cell-mediated or antibody-mediated. The vasculature components are targeted, and the histopathologic changes depend on the organ involved.

Amyloidosis 8

Learning Objectives

- ❑ Answer questions about composition of amyloid
- ❑ Explain information related to systemic types of amyloid
- ❑ Demonstrate understanding of localized types of amyloid

COMPOSITION OF AMYLOID

Amyloidosis is a group of diseases characterized by the deposition of an extracellular protein that has specific properties.

- Individual molecular subunits form β-pleated sheets. Amorphous eosinophilic extracellular deposits of amyloid are seen on the H&E stain. These deposits stain red with the Congo red stain, and apple green birefringence of the amyloid is seen on the Congo red stain under polarized light.
- The fibrillary protein of amyloid varies with each disease. Also present in amyloid are serum amyloid P (SAP) and glycosaminoglycans (heparan sulfate).

SYSTEMIC TYPES OF AMYLOID

Primary amyloidosis has amyloid light chain (AL) amyloid, whose fibrillary protein is made of kappa or lambda light chains. Primary amyloidosis may be seen in plasma cell disorders (multiple myeloma, B-cell lymphomas, etc.) but most cases occur independent of other diseases.

Reactive systemic amyloidosis (secondary amyloidosis) has amyloid-associated (AA) protein, whose precursor is serum amyloid A (SAA), an acute phase reactant produced by the liver which is elevated with ongoing chronic inflammation and neoplasia. Reactive systemic amyloidosis can be seen with a wide variety of chronic diseases, including rheumatoid arthritis, systemic lupus erythematosus, tuberculosis, bronchiectasis, osteomyelitis, inflammatory bowel disease, and cancer.

Familial Mediterranean fever has AA type amyloid with fibrillary protein composed of serum amyloid A (SAA). This autosomal recessive disease is characterized by recurrent inflammation, fever, and neutrophil dysfunction. Gain of function mutations of *pyrin* are present.

Hemodialysis-associated amyloidosis has Aβ2M type amyloid with precursor protein β2-microglobulin. This form of amyloidosis may cause carpal tunnel syndrome and joint disease.

Clinical Correlate

Carpal tunnel syndrome is caused when fibrosis, edema, or another pathologic process compresses and damages the median nerve within the tunnel formed by the carpal bones and flexor retinaculum.

TTR = transporter of thyroxine and retinol

LOCALIZED TYPES OF AMYLOID

Senile cerebral amyloidosis (Alzheimer disease) has Aβ type amyloid with fibrillary protein composed of β-amyloid precursor protein (βAPP). It is found in Alzheimer plaques and in cerebral vessels. The gene for βAPP is located on chromosome 21.

Senile cardiac/systemic amyloidosis has ATTR type amyloid with fibrillary protein composed of transthyretin. This type of amyloidosis is seen in men older than 70 years and may cause heart failure as a result of restrictive/infiltrative cardiomyopathy. Four percent of African Americans have a transthyretin (TTR) V1221 mutation with 1% being homozygous, serving as a risk for cardiac disease.

Endocrine type amyloidosis is seen in medullary carcinoma of the thyroid (procalcitonin), adult-onset diabetes (amylin), and pancreatic islet cell tumors (amylin).

CLINICAL FEATURES

In **systemic forms** of amyloidosis, the kidney is the most commonly involved organ, and patients may experience nephrotic syndrome and/or progressive renal failure. Cardiac involvement may cause restrictive cardiomyopathy and conduction disturbances. Other clinical features include hepatosplenomegaly and involvement of the gastrointestinal tract, which may produce tongue enlargement (macroglossia, primarily in AL type) and malabsorption.

Diagnosis in systemic forms of amyloidosis can be established with biopsy of the rectal mucosa, gingiva, or the abdominal fat pad; Congo red stain shows apple green birefringence under polarized light of amyloid deposits. The prognosis of systemic amyloidosis is poor. AL amyloidosis is diagnosed by serum and urinary protein electrophoresis and immunoelectrophoresis. Proteomic analysis is another diagnostic tool.

Principles of Neoplasia 9

Learning Objectives

- ❑ Use knowledge of epidemiology of neoplasias
- ❑ Answer questions about carcinogenic agents
- ❑ Solve problems concerning carcinogenesis
- ❑ Answer questions about diagnosis of cancer

DEFINITION

In **neoplasia**, an abnormal cell or tissue grows more rapidly than normal cells or tissue; it does so by acquiring multiple genetic changes over time and by continuing to grow after the stimuli that initiated the new growth have been removed.

EPIDEMIOLOGY

Cancer is the **second leading cause of death** in the United States. In 2015, the estimated number of new cancers diagnosed was 1,658,370, and the estimated number of deaths from cancer was 589,430.

In men, the sites with the highest new cancer rates are (in order of decreasing frequency):

- Prostate
- Lung and bronchus
- Colon and rectum

These same sites have the highest mortality rate, although lung and bronchus cancers more commonly cause death than prostate cancer.

In women, the sites with the highest new cancer rates are (in order of decreasing frequency):

- Breast
- Lung and bronchus
- Colon and rectum

These same sites have the highest mortality rate, although lung and bronchus cancers more commonly cause death than breast cancer.

In children, the most common cancers are acute lymphocytic leukemia, CNS malignancy, neuroblastoma, and non-Hodgkin lymphoma.

Predisposition to cancer involves many factors. Geographic and racial factors can be important:

- Stomach cancer is **much more prevalent** in Japan than in the United States.
- Breast cancer is **much more prevalent** in the United States than in Japan.
- Liver hepatoma is **much more prevalent** in Asia than in the United States.
- Prostate cancer is **more prevalent** in African Americans than in Caucasians.

Hereditary predisposition can be seen in many cancers, including familial retinoblastoma, multiple endocrine neoplasia, and familial polyposis coli.

Acquired preneoplastic disorders also affect cancer incidence, with examples including cervical dysplasia (characterized by changes in cell size and shape), endometrial hyperplasia, cirrhosis, inflammatory bowel disease, and chronic atrophic gastritis.

CARCINOGENIC AGENTS

Chemical carcinogens. Carcinogenesis is a multistep process involving a sequence of initiation (mutation) followed by promotion (proliferation). Initiators can be either direct-acting chemical carcinogens (mutagens which cause cancer directly by modifying DNA) or indirect-acting chemical carcinogens (procarcinogens which require metabolic conversion to form active carcinogens). Promotors cause cellular proliferation of mutated (initiated) cells, which may lead to accumulation of additional mutations.

- **Clinically important chemical carcinogens** are numerous, and include nitrosamines (gastric cancer), cigarette smoke (multiple malignancies), polycyclic aromatic hydrocarbons (bronchogenic carcinoma), asbestos (bronchogenic carcinoma, mesothelioma), chromium and nickel (bronchogenic carcinoma), arsenic (squamous cell carcinomas of skin and lung, angiosarcoma of liver), vinyl chloride (angiosarcoma of liver), aromatic amines and azo dyes (hepatocellular carcinoma), alkylating agents (leukemia, lymphoma, other cancers), benzene (leukemia), and naphthylamine (bladder cancer). Potential carcinogens are screened by the Ames test, which detects any mutagenic effects of potential carcinogens on bacterial cells in culture; mutagenicity *in vitro* correlates well with carcinogenicity in vivo.

Bridge to Biochemistry

Diseases associated with DNA repair include xeroderma pigmentosum and hereditary nonpolyposis colorectal cancer.

Radiation. Ultraviolet B sunlight is the most carcinogenic because it produces pyrimidine dimers in DNA, leading to transcriptional errors and mutations of oncogenes and tumor suppressor genes, thereby increasing the risk of skin cancer. Xeroderma pigmentosum is an autosomal recessive inherited defect in DNA repair, in which the pyrimidine dimers formed with ultraviolet B sunlight cannot be repaired; this defect predisposes to skin cancer. Ionizing radiation includes x-rays and gamma rays, alpha and beta particles, protons, and neutrons. Cells in mitosis or the G2 phase of the cell cycle are most sensitive to radiation. Radiation causes cross-linking and chain breaks in nucleic acids. Atomic bomb survivors experienced an increased incidence of leukemias, thyroid cancer, and other cancers. Uranium miners historically had increased lung cancer, related to inhalation of radioactive radon, which is a decay product of uranium.

Oncogenic viruses.

RNA oncogenic viruses. Human T-cell leukemia virus (HTLV-1) causes adult T-cell leukemia/lymphoma.

DNA oncogenic viruses include the following:

- Hepatitis B virus (hepatocellular carcinoma)
- Epstein-Barr virus (EBV), which has been implicated in Burkitt lymphoma, B-cell lymphomas in immunosuppressed patients, nasopharyngeal carcinoma
- Human papilloma virus (HPV), which causes benign squamous papillomas (warts-condyloma acuminatum) and a variety of carcinomas (cervical, vulvar, vaginal, penile, and anal)
- Kaposi-sarcoma-associated herpesvirus (HHV8) which causes Kaposi sarcoma

Loss of immune regulation. Immunosurveillance normally destroys neoplastic cells via recognition of "non-self" antigens, and both humoral and cell-mediated immune responses play a role. Patients with immune system dysfunction have an increased number of neoplasms, especially malignant lymphomas.

CARCINOGENESIS

Carcinogenesis is a multistep process, and development of all human cancers appears to require the accumulation of multiple genetic changes. These changes can involve either inherited germline mutations or acquired mutations. Once a single severely mutated cell forms, monoclonal expansion of the cell's line can cause a tumor. Most important mutations in tumorigenesis involve growth promoting genes (proto-oncogenes), growth inhibiting tumor suppressor genes, or the genes regulating apoptosis and senescence.

Activation of growth promoting oncogenes. Proto-oncogenes are normal cellular genes involved with growth and cellular differentiation. Oncogenes are derived from proto-oncogenes by either a change in the gene sequence, resulting in a new gene product (oncoprotein), or a loss of gene regulation resulting in overexpression of the normal gene product. Mechanisms of oncogene activation include point mutations, chromosomal translocations, gene amplification, and insertional mutagenesis. Activated oncogenes lack regulatory control and are overexpressed, resulting in unregulated cellular proliferation.

Table 9-1. Clinically Important Oncogenes

Oncogene	Tumor	Gene Product	Mechanism of Activation
FGF3 & *FGF4*	Cancer of the stomach, breast, bladder, and Kaposi sarcoma	**Growth factors** Fibroblast growth factor	Overexpression
PDGFRA	Astrocytoma	Platelet-derived growth factor	Overexpression
ERBB1	Squamous cell carcinoma of lung	**Growth factor receptors** Epidermal growth factor receptor	Overexpression
ERBB2	Breast, ovary, lung	Epidermal growth factor receptor	Amplification
ERBB3	Breast	Epidermal growth factor receptor	Overexpression
RET	MEN 2A & 2B, familial thyroid (medullary) cancer	Glial neurotrophic factor receptor	Point mutation
ABL	CML, ALL	**Signal transduction proteins** bcr-abl fusion protein with tyrosine kinase activity	Translocation t(9;22)
KRAS	Lung, pancreas, and colon	GTP binding protein	Point mutation
MYC	Burkitt lymphoma	Nuclear regulatory protein	Translocation t(8;14)
MYCL	Small cell lung carcinoma	Nuclear regulatory protein	Amplification
MYCN	Neuroblastoma	Nuclear regulatory protein	Amplification
CCND1	Mantle cell lymphoma	**Cell cycle regulatory proteins** Cyclin D protein	Translocation t(11;14)
CDK4	Melanoma, GBM	Cyclin dependent kinase	Amplification

Inactivation of tumor suppressor genes. Tumor suppressor genes encode proteins that regulate and suppress cell proliferation by inhibiting progression of the cell through the cell cycle. The mechanism of action of tumor suppressor genes may vary. As examples, p53 prevents a cell with damaged DNA from entering S-phase, while Rb prevents the cell from entering S-phase until the appropriate growth signals are present.

- **Knudson's "two hit hypothesis"** states that at least 2 tumor suppressor genes must be inactivated for oncogenesis. In cancers arising in individuals with inherited germline mutations, the "first hit" is the inherited germline mutation and the "second hit" is an acquired somatic mutation. Examples of inherited germline mutations include familial retinoblastoma (in which germline mutation of *RB1* on chromosome 13 is associated with a high rate of retinoblastoma and osteosarcoma) and Li-Fraumeni syndrome (in which germline mutation of *TP53* on chromosome 17 is associated with a high rate of many types of tumors).

Table 9-2. Clinically Important Tumor Suppressor Genes

Chromosome	Gene	Tumors
3p25.3	*VHL*	von Hippel-Lindau disease, renal cell carcinoma
11p13	*WT1*	Wilms tumor
11p15.5	*WT2*	Wilms tumor
13q14.2	*RB1*	Retinoblastoma, osteosarcoma
17p13.1	*TP53*	Lung, breast, colon, and others
17q21.31	*BRCA1*	Hereditary breast and ovary cancer
13q13.1	*BRCA2*	Hereditary breast cancer
5q22.2	*APC*	Adenomatous polyps and colon cancer
18q21.2	*DCC*	Colon cancer
17q11.2	*NF1*	Neurofibromas
22q12.2	*NF2*	Acoustic neuromas, meningiomas

Regulation of apoptosis. Tumor genesis related to changes in regulation of apoptosis occurs in the follicular lymphomas that have the translocation t(14;18). Normally, Bcl-2 prevents apoptosis (programmed cell death). In the follicular lymphomas with this translocation, the Bcl-2 regulator of apoptosis is overexpressed, because the translocation connects the immunoglobulin heavy chain gene on chromosome 14 (which turns on easily in B lymphocytes) to the *BCL2* gene on chromosome 18, thereby leading to a situation in which lymphocytes fail to die as expected and instead produce a tumor.

Other examples of apoptosis regulators include Bax, Bad, bcl-xS, and Bid; p53 promotes apoptosis in mutated cells by stimulating bax synthesis. The protein c-myc promotes cellular proliferation and when associated with p53 leads to apoptosis and when associated with Bcl-2 inhibits apoptosis.

Limitless replication is possible due in part to upregulation of telomerase.

Sustained angiogenesis is possible due in part to activation of the Notch signaling pathway.

Invasiveness/metastasis. Malignant cells must dissociate from tumors (loss of E-cadherin function) and degrade the extracellular matrix before spreading to distant sites. Cancer-associated glycans are being investigated for their role in cancer spread and as targets for therapy.

DIAGNOSIS OF CANCER

Table 9-3. General Features of Benign versus Malignant Neoplasms

	Benign	Malignant
Gross	• Small size • Slow growing • Encapsulated or well-demarcated borders	• Larger in size • Rapid growth • Necrosis and hemorrhage are commonly seen • Poorly demarcated
Micro	• Expansile growth with well-circumscribed borders • Tend to be well differentiated • Resemble the normal tissue counterpart from which they arise • Noninvasive and never metastasize	• Vary from well to poorly (anaplastic) differentiated • Tumor cells vary in size and shape (pleomorphism) • Increased nuclear to cytoplasmic ratios • Nuclear hyperchromasia and prominent nucleoli • High mitotic activity with abnormal mitotic figures • **Invasive growth pattern** • **Has potential to metastasize**

Histologic diagnosis of cancer. Microscopic examination of tissue or cells is required to make the diagnosis of cancer. Material suitable for diagnosis of a tumor may be obtained by complete excision, biopsy, fine needle aspiration, or cytologic smears (Pap test).

- **Immunohistochemistry** may be helpful in confirming the tissue of origin of metastatic or poorly differentiated tumors. The technique uses monoclonal antibodies that are specific for a cellular component. Among the many antibodies that are clinically useful are:
  - All of the serum tumor markers
  - Thyroglobulin (thyroid cancers)
  - S100 (melanoma and neural tumors)
  - Actin (smooth and skeletal muscle)
  - CD markers (lymphomas/leukemias)
  - Estrogen receptors (breast cancer)
  - Intermediate filaments

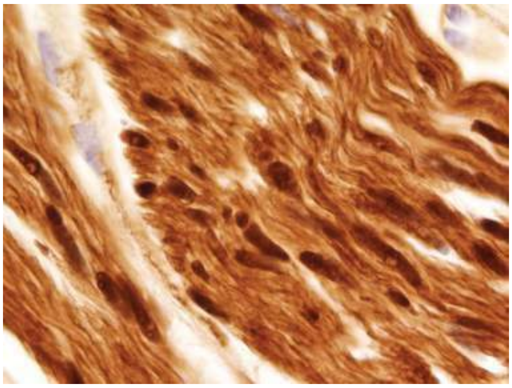

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Figure 9-1. S100 Staining of a Neurofibroma

- **Ancillary tests** for the diagnosis of cancer include electron microscopy, flow cytometry, cytogenetics, and PCR/DNA probes.

Table 9-4.
Expression of Intermediate Filaments by Normal and Malignant Cells

Intermediate Filament	Normal Tissue Expression	Tumor
Keratin	All epithelial cells	Carcinomas
Vimentin	Mesenchymal cells	Sarcomas
Desmin	Muscle cells	Uterine leiomyoma Rhabdomyosarcoma
Neurofilament	CNS and PNS neurons Neural crest derivatives	Pheochromocytoma Neuroblastoma
Glial fibrillary acidic protein (GFAP)	Glial cells	Astrocytomas Ependymomas

Serum tumor markers. Tumor markers are usually normal cellular components that are increased in neoplasms but may also be elevated in nonneoplastic conditions. Serum tumor markers are used for screening (e.g., prostate specific antigen [PSA]) for cancer, monitoring treatment efficacy, and detecting recurrence of cancers.

- **Clinically useful tumor markers** include alpha-fetoprotein (AFP, used for hepatoma, nonseminomatous testicular germ cell tumors); beta human chorionic gonadotropin (hCG, used for trophoblastic tumors, choriocarcinoma); calcitonin (used for medullary carcinoma of the thyroid); carcinoembryonic antigen (CEA, used for carcinomas of the lung, pancreas, stomach, breast, and colon); CA-125 (used for malignant ovarian epithelial tumors); CA19-9 (used for malignant pancreatic adenocarcinoma); placental alkaline phosphatase (used for seminoma); and prostate specific antigen (PSA, used for prostate cancer).

Grading and staging. Tumor grade is a histologic estimate of the malignancy of a tumor, and typically uses criteria such as the degree of differentiation from low grade (well-differentiated) to high grade (poorly differentiated/anaplastic) and the number of mitoses.

Tumor stage is a clinical estimate of the extent of tumor spread. TNM staging system criteria are used for most tumor types:

- **T** indicates the size of the primary tumor.
- **N** indicates extent of regional lymph node spread.
- **M** indicates the presence or absence of metastatic disease.

In general, staging is a better predictor of prognosis than tumor grade.

Note

Most neoplasms (90%) arise from epithelium, with the remainder from mesenchymal cells.

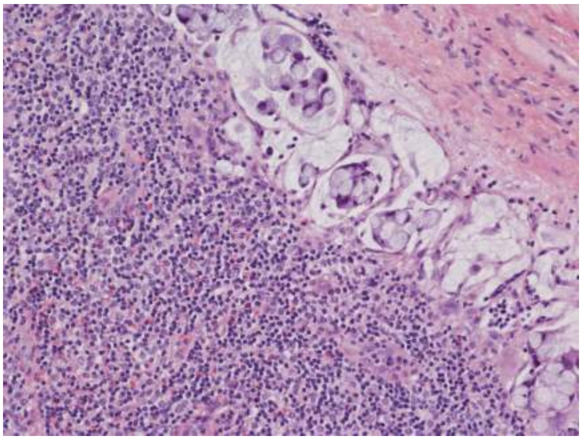

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Figure 9-2. Involvement of Lymph Node by Signet Ring Cell Carcinoma

Tumor progression refers to the tendency of a tumor to become more malignant over time. This progression can be related to both natural selection (evolution of a more malignant clone over time due to a selective growth advantage) and genetic instability (malignant cells are more prone to mutate and accumulate additional genetic defects).

Metastasis. Lymphatic spread is the most common initial route of spread for epithelial carcinomas. Early hematogenous spread is typically seen with most sarcomas (e.g., osteogenic sarcoma), renal cell carcinoma (because of the proximity of the large renal vein), hepatocellular carcinoma (because of the presence of the hepatic sinusoids), follicular carcinoma of the thyroid, and choriocarcinoma (because of its propensity to seek vessels). Seeding of body cavities and surfaces occurs in ovarian carcinoma. Transplantation via mechanical manipulation (e.g., surgical incision, needle tracts) may occur but is relatively rare.

Skin Pathology 10

Learning Objectives

- ❑ Solve problems concerning disorders of pigmentation
- ❑ Answer questions about melanocytic tumors
- ❑ Explain information related to epidermal and dermal lesions
- ❑ Explain information related to malignant tumors

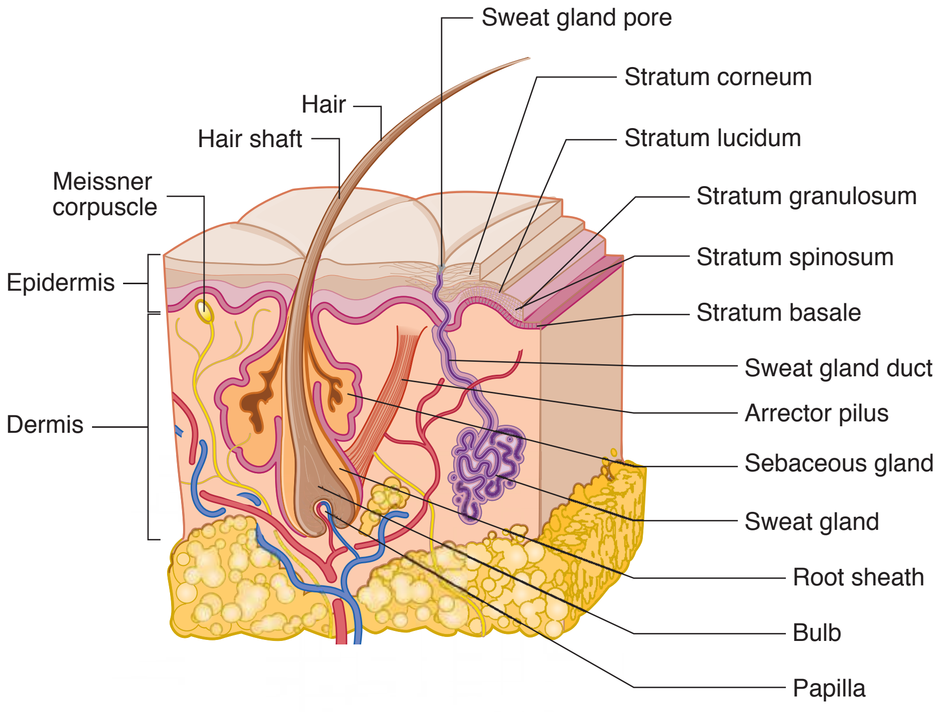

Figure 10-1. Skin

DISORDERS OF PIGMENTATION

Vitiligo causes irregular, completely depigmented skin patches. It is common and can affect any race; there may also be a familial predisposition. The disease has an unknown etiology that is possibly autoimmune. Microscopically, affected areas are devoid of epidermal melanocytes.

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Figure 10-2. Vitiligo

Melasma causes irregular blotchy patches of hyperpigmentation on the face; it is associated with sun exposure, oral contraceptive use, and pregnancy ("mask of pregnancy") and may regress after pregnancy.

Freckles (ephelides) are light brown macules on the face, shoulders, and chest. They are common in fair-skinned children and tend to darken and fade with the seasons due to sunlight exposure. Microscopically, freckles are characterized by increased melanin deposition in the basal cell layer of the epidermis with a normal number of melanocytes.

Benign **lentigo** is a localized proliferation of melanocytes which cause small, oval, light brown macules. Microscopically, benign lentigos show linear melanocytic hyperplasia.

MELANOCYTIC TUMORS

Congenital nevi (birthmarks) are present at birth; giant congenital nevi have increased risk of developing melanoma.

Nevocellular nevus (mole) is a benign tumor of melanocytes (melanocytic nevus cells) that is clearly related to sun exposure. Types of nevi include junctional, compound, and intradermal. Nevi have uniform tan to brown color with sharp, well-circumscribed borders and tend to be stable in shape and size. Malignant transformation is uncommon.

Dysplastic nevi (BK moles) are larger and more irregular than common nevi, and they may have pigment variation. Microscopically, the nevus exhibits cytological and architectural atypia. Dysplastic nevus syndrome is autosomal dominant (*CMM1* locus on chromosome 1); patients often have multiple dysplastic nevi; and there is increased risk of developing melanoma.

Malignant melanoma is a malignancy of melanocytes whose incidence is increasing at a rapid rate, with peak in ages 40–70. Risk factors include chronic sun exposure, sunburn, fair skin, dysplastic nevus syndrome, and familial melanoma (associated with loss of function mutation of the p16 tumor suppressor gene, *CDKN2A*, on chromosome 9; somatic mutations of *NRAS* and *BRAF* also occur). Melanomas characteristically form skin lesions of large diameter with asymmetric and irregular borders and variegated color; the lesions may be macules,

papules, or nodules. Melanomas on males have increased frequency on the upper back; females have increased frequency on the back and legs.

Several **types of melanomas** occur:

- Lentigo maligna melanoma is usually located on the face or neck of older individuals and has the best prognosis.
- Superficial spreading melanoma is the most common type of melanoma and has a primarily horizontal growth pattern.
- Acral lentiginous melanoma is the most common melanoma in dark-skinned individuals; it affects palms, soles, and subungual area.
- Nodular melanoma is a nodular tumor with a vertical growth pattern that has the worst prognosis of the melanomas.

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Figure 10-3. Melanoma
(Note the size, irregular borders, and variegated color)

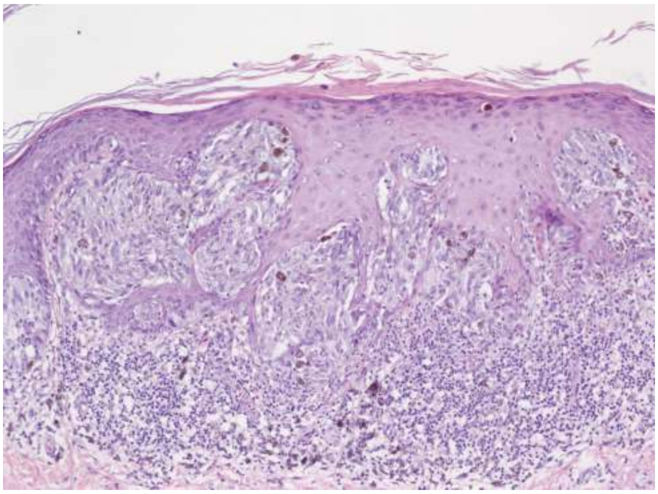

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Figure 10-4. Malignant Melanoma
(Note nests of malanocytes)

The **prognosis** of melanomas is determined by TNM staging; T status is based on the depth of invasion (Breslow thickness measured histologically in millimeters).

Local disease is treated with wide surgical excision and sometimes sentinel node biopsy. Systemic disease is treated with chemotherapy or immunotherapy. Metastases may occur after years of dormancy.

EPIDERMAL AND DERMAL LESIONS

Acanthosis nigricans causes thickened, hyperpigmented skin of the posterior neck, axillae, and groin; it is often associated with obesity and hyperinsulinism. On rare occasions it is associated with internal malignancy (stomach and other gastrointestinal malignancies).

Seborrheic keratoses are benign squamoproliferative neoplasms that are very common in middle-aged and elderly individuals; they may occur on the trunk, head, neck, and the extremities. The lesions are tan to brown coin-shaped plaques

that have a granular surface with a “stuck on” appearance, characterized microscopically by basaloid epidermal hyperplasia and “horn cysts” (keratin-filled epidermal pseudocysts). They are usually left untreated, but may be removed if they become irritated or for cosmetic purposes. The sign of Leser-Trélat (paraneoplastic syndrome) is the sudden development of multiple lesions which may accompany an internal malignancy.

Psoriasis is an autoimmune disorder with a clear genetic component that causes increased proliferation and turnover of epidermal keratinocytes; it affects 1% of the U.S. population. The most common form is psoriasis vulgaris. Common sites of involvement include the knees, elbows, and scalp; the classic skin lesion is a well-demarcated erythematous plaque with a silvery scale. Removal of scale results in pinpoint bleeding (Auspitz sign). Nail beds show pitting and discoloration. Psoriasis may be associated with arthritis, enteropathy, and myopathy.

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Figure 10-5. The Silvery Plaques of Psoriasis

- **Microscopically**, the lesions show epidermal hyperplasia (acanthosis), patchy hyperkeratinization with parakeratosis, uniform elongation and thickening of the rete ridges, thinning of the epidermis over the dermal papillae, and Munro microabscesses.
- Treatment is topical steroids and ultraviolet irradiation; severe systemic disease may be treated with methotrexate.

Pemphigus is a rare, potentially fatal autoimmune disorder that is characterized by intraepidermal blister formation. Pemphigus vulgaris is the most common form. The pathogenesis involves the production of autoantibodies directed against a part of the keratinocyte desmosome called desmoglein 3, with resulting loss of intercellular adhesion (acantholysis) and blister formation. Pemphigus causes mucosal lesions and easily ruptured, flaccid blisters. Oral involvement is common.

- Microscopic examination shows intraepidermal acantholysis; the acantholysis leaves behind a basal layer of keratinocytes, which has a tombstone-like arrangement. Immunofluorescence shows a net-like pattern of IgG staining between the epidermal keratinocytes that create bullae.
- Treatment is with immunosuppression.

Bullous pemphigoid is a relatively common autoimmune disorder of older individuals characterized by subepidermal blister formation with tense bullae that do not rupture easily. The condition results from production of autoantibodies directed against a part of the keratinocyte hemidesmosome called *bullous pemphigoid antigens 1 and 2*. Immunofluorescence shows linear deposits of IgG at the dermal-epidermal junction.

Dermatitis herpetiformis is a rare immune disorder that is often associated with celiac sprue; it is characterized by subepidermal blister formation with itchy, grouped vesicles and occasional bullae on the extensor surfaces. Production of IgA antibodies directed against gliadin and other antigens deposit in the tips of the dermal papillae and result in subepidermal blister formation. Routine microscopy shows microabscesses at the tips of the dermal papillae that can lead to eventual subepidermal separation results in blister formation; immunofluorescence shows granular IgA deposits at the tips of the dermal papillae. Dermatitis herpetiformis often responds to a gluten-free diet.

Porphyria cutanea tarda is an acquired and familial disorder of heme synthesis. Patients experience upper extremity blistering secondary to sun exposure and minor trauma. Microscopically, there are subepidermal blisters with minimal inflammation. Dermal vessels are thickened. Direct immunofluorescence shows deposition of immunoglobulins and complement at the epidermal basement membrane and around dermal vessels.

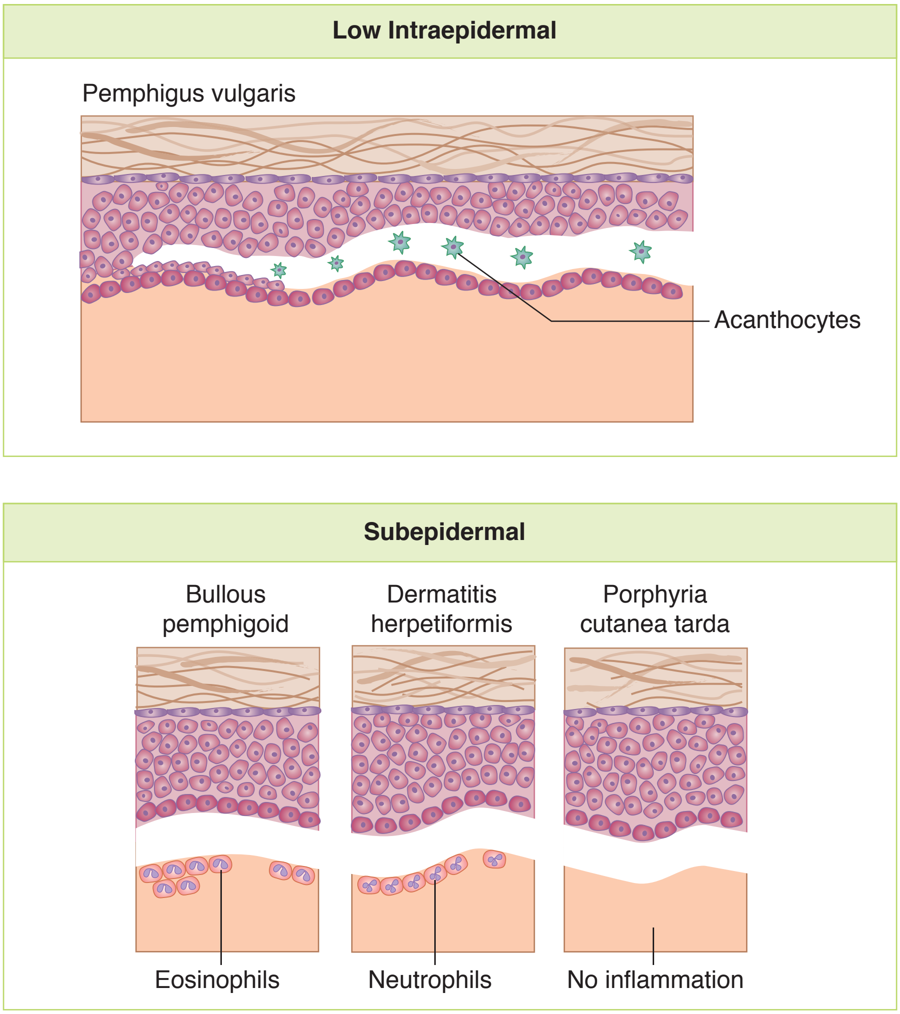

Figure 10-6. Intraepidermal and Subepidermal Blisters

Ichthyosis vulgaris is a common inherited (autosomal dominant) skin disorder characterized by a thickened stratum corneum with absent stratum granulosum. Onset is in childhood. Patients have hyperkeratotic, dry skin on the trunk and extensor surfaces of limb areas.

Xerosis is a common cause of pruritus and dry skin in the elderly that is due to decreased skin lipids. Cancer patients receiving epidermal growth factor receptor inhibitor are susceptible. Treatment is with emollients.

Eczema is a group of related inflammatory skin diseases characterized by pruritus and epidermal spongiosis (edema).

- Acute eczema causes a vesicular, erythematous rash.
- Chronic eczema develops following repetitive scratching, and is characterized by dry, thickened, hyperkeratotic skin.
- Atopic dermatitis is often inherited. Defects in the keratinocyte barrier are due to mutations in the filaggrin gene (*FLG*).
- Contact dermatitis can be either allergic type (poison ivy, nickel in jewelry) or photodermatitis type (such as photosensitivity reaction after tetracycline).

Polymorphous light eruption is the most common idiopathic form of photodermatosis and causes pruritic erythematous macules, papules, plaques, or vesicles on exposure to sunlight. There is dermal edema and inflammation.

Verrucae (warts) are caused by human papillomavirus. Verruca vulgaris is the most common type.

Cutaneous lupus erythematosus may be acute (facial butterfly rash), subacute (photosensitive rash on anterior chest, upper back and upper extremities), or chronic (discoid plaques, usually above the neck). Direct immunofluorescence shows deposition of immunoglobulin and complement at the dermal-epidermal junction. Serologies for autoantibodies and clinical correlation help establish the diagnosis.

Erythema multiforme is a hypersensitivity skin reaction to infections (*Mycoplasma pneumoniae*, herpes simplex) or drugs (sulfonamides, penicillin, barbiturates, phenytoin) characterized by vesicles, bullae, and "targetoid" erythematous lesions. The most severe form is Stevens-Johnson syndrome, which has extensive involvement of skin and mucous membranes.

Pityriasis rosea causes a pruritic rash that starts with an oval-shaped "herald patch" and progresses to a papular eruption of the trunk to produce a "Christmas tree" distribution. It is clinically diagnosed, self-limiting and possibly a viral exanthem.

Granuloma annulare is a chronic inflammatory disorder that causes papules and plaques. Palisaded granulomas are present microscopically. The pathogenesis is immunologic, but most cases occur in healthy patients.

Erythema nodosum causes raised, erythematous, painful nodules of subcutaneous adipose tissue, typically on the anterior shins, which can be associated with granulomatous diseases and streptococcal infection.

Epidermoid cyst is a common benign skin cyst lined with stratified squamous epithelium and filled with keratin debris.

MALIGNANT TUMORS

Squamous cell carcinoma (SCC) has peak incidence at age 60. Risk factors include chronic sun exposure (ultraviolet UVB); fair complexion; chronic skin ulcers or sinus tracts; long-term exposure to hydrocarbons, arsenic, burns, and radiation; immunosuppression; and xeroderma pigmentosum. *TP53* and *HRAS* mutations are common.

- Precursors include actinic keratosis (a sun-induced dysplasia of the keratinocytes that causes rough, red papules on the face, arms, and hands) and Bowen disease (squamous cell carcinoma *in situ*).
- Squamous cell carcinoma occurs on sun-exposed areas (face and hands) and causes a tan nodular mass which commonly ulcerates. Microscopic examination shows nests of atypical keratinocytes that invade the dermis, (oftentimes) formation of keratin pearls, and intercellular bridges (desmosomes) between tumor cells. Squamous cell carcinoma of the skin rarely metastasizes and complete excision is usually curative.
- A variant is **keratoacanthoma** (well differentiated SCC), which causes rapidly growing, dome-shaped nodules with a central keratin-filled crater; these are often self-limited and may regress spontaneously.

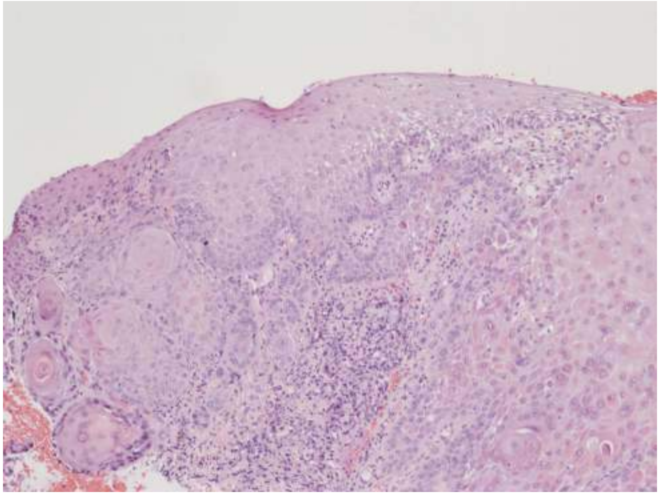

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Figure 10-7. Squamous Cell Carcinoma
(Note the keratin pearls)

Basal cell carcinoma (BCC) is the **most common tumor in adults in the Western world**; it is most common in middle-aged or elderly individuals and arises from the basal cells of hair follicles. Risk factors include chronic sun exposure, fair complexion, immunosuppression, and xeroderma pigmentosum.

BCC occurs on sun-exposed, hair-bearing areas (face), and may form pearly papules; nodules with heaped-up, translucent borders, telangiectasia, or ulcers (rodent ulcer). Microscopically, it shows invasive nests of basaloid cells with a palisading growth pattern.

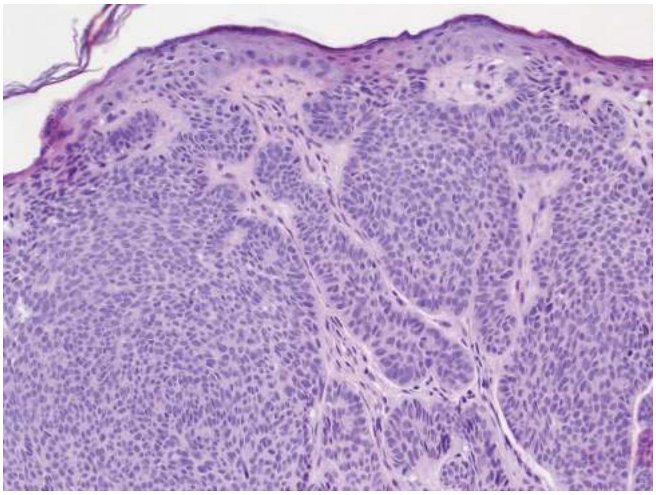

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Figure 10-8. Basal Cell Carcinoma

BCC grows slowly and rarely metastasizes, but it may be locally aggressive. Shave biopsies have a 50% recurrence rate, but complete excision is usually curative. Mutations affecting the Hedgehog pathway are seen in sporadic and familial cases.

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Figure 10-9. Pearly Papule Characteristic of a Basal Cell Carcinoma

Red Blood Cell Pathology: Anemias 11

Learning Objectives

- ❑ Explain information related to red blood cell morphology
- ❑ Solve problems using knowledge of microcytic, normocytic, and macrocytic anemias
- ❑ Demonstrate understanding of polycythemia vera

RED BLOOD CELL MORPHOLOGY

Red Cell Shapes

Abnormal size is called anisocytosis (*aniso* means unequal). Abnormal shape is called poikilocytosis (*poikilo* means various). **Elliptocytes** may be seen in hereditary elliptocytosis. **Spherocytes** result from decreased erythrocyte membrane, and they may be seen in hereditary spherocytosis and in autoimmune hemolytic anemia. **Target cells** result from increased erythrocyte membrane, and they may be seen in hemoglobinopathies, thalassemia, and liver disease. **Acanthocytes** have irregular spicules on their surfaces; numerous acanthocytes can be seen in abetalipoproteinemia. **Echinocytes** (burr cells) have smooth undulations on their surface; they may be seen in uremia or more commonly as an artifact.

Schistocytes are erythrocyte fragments (helmet cells are a type of schistocyte); they can be seen in microangiopathic hemolytic anemias or traumatic hemolysis. **Bite cells** are erythrocytes with "bites" of cytoplasm being removed by splenic macrophages; they may be seen in G6PD deficiency. **Teardrop cells** (dacrocytes) may be seen in thalassemia and myelofibrosis. **Sickle cells** (drepanocytes) are seen in sickle cell anemia. **Rouleaux** ("stack of coins") refers to erythrocytes lining up in a row. Rouleaux are characteristic of multiple myeloma.

Red Cell Inclusions

Basophilic stippling results from cytoplasmic remnants of RNA; it may indicate reticulocytosis or lead poisoning. **Howell-Jolly bodies** are remnants of nuclear chromatin that may occur in severe anemias or patients without spleens. **Pappenheimer bodies** are composed of iron, and they may be found in the peripheral blood following splenectomy. **Ring sideroblasts** have iron trapped abnormally in mitochondria, forming a ring around nucleus; they can be seen in sideroblastic anemia. **Heinz bodies** result from denatured hemoglobin; they can be seen with glucose-6-phosphate dehydrogenase deficiency.

Bridge to Physiology

Patients with anemia have normal SaO_2 and PaO_2, but they have reduced oxygen content due to the low level of hemoglobin.

ANEMIAS

Anemia is a reduction below normal limits of the total circulating red cell mass. Signs of anemia include palpitations, dizziness, angina, pallor of skin and nails, weakness, claudication, fatigue, and lethargy.

- **Reticulocytes** are immature, larger red cells (macrocytic cells) that are spherical and have a bluish color (polychromasia) due to free ribosomal RNA. Reticulocytes do not have a nucleus; note that any erythrocyte with a nucleus (nRBC) in peripheral blood is abnormal. Reticulocyte maturation to a mature erythrocyte takes about 1 day. The reticulocyte count is the percentage of red immature cells present in peripheral blood (normal 0.5–1.5%).

  The corrected reticulocyte count takes into consideration the degree of anemia and is calculated as (patient's hct/45) × (reticulocyte count); the idea behind the calculation is to scale the reticulocyte count by multiplying by the ratio of the patient's hematocrit to "normal" hematocrit of 45%. When interpreting the corrected reticulocyte count, <2% indicates poor bone marrow response and >3% indicates good bone marrow response.

- The reticulocyte production index is the corrected reticulocyte count/2; use this measure if bone marrow reticulocytes (shift cells) are present (polychromasia). The division by 2 is because shift cells take twice as long as reticulocytes to mature (2 days versus 1 day).

Laboratory terms used with respect to the population of erythrocytes:

- **Mean corpuscular volume** (MCV): average volume of a red blood cell
- **Mean corpuscular hemoglobin** (MCH): average content (mass) of hemoglobin per cell
- **Mean corpuscular hemoglobin concentration** (MCHC): average concentration of hemoglobin in a given volume of packed erythrocytes
- **Red cell distribution width** (RDW): coefficient of variation of red blood cell volume and a measure of anisocytosis

Classification of anemia can be based on color: normochromic anemias have normal red cell color (central pallor of about a third the diameter of the erythrocyte); hypochromic anemias have decreased color (seen as an increased central pallor of erythrocyte); and hyperchromic anemias, while theoretically possible, are usually instead called spherocytosis and have increased color (loss of central pallor of erythrocyte). Classification of anemia can also be based on size (MCV).

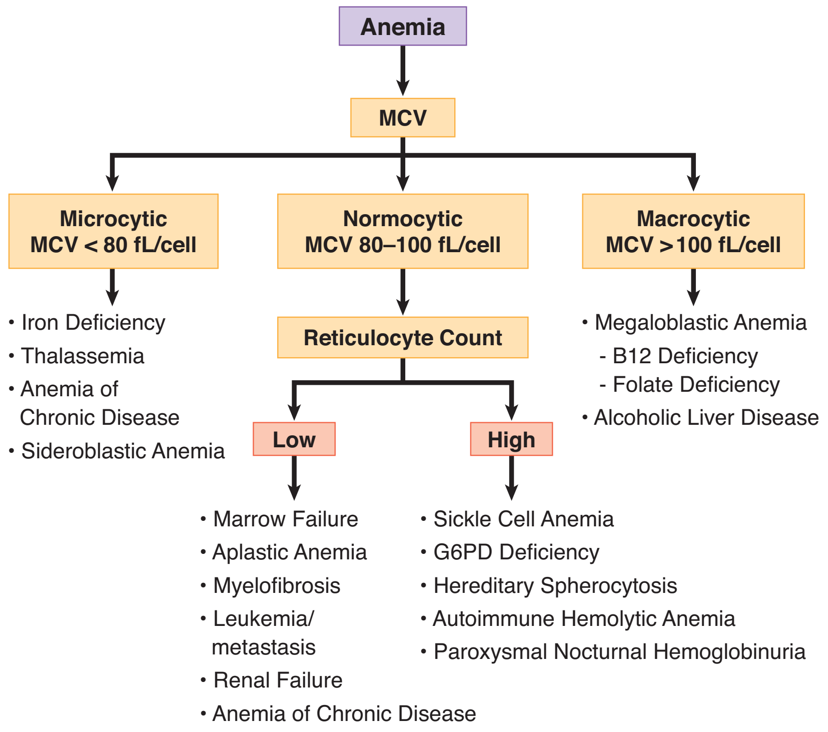

Figure 11-1. Classification of Anemias Based on MCV

The pathogenesis of anemia varies with the underlying disease. Blood loss can cause anemia. **Hemolytic anemias** are also important, and include hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, sickle cell disease, hemoglobin C disease, thalassemia, and paroxysmal nocturnal hemoglobinuria. **Immunohemolytic anemias**, which are hemolytic anemias with an immune component to the pathology, include autoimmune hemolytic anemia (AIHA), cold AIHA, incompatible blood transfusions, and hemolytic disease of the newborn. **Anemias of diminished erythropoiesis** include megaloblastic anemia (B12 and folate deficiencies), iron deficiency anemia, anemia of chronic disease, aplastic anemia, myelophthisic anemia, and sideroblastic anemia.

MICROCYTIC ANEMIAS

Iron Deficiency Anemia

Iron physiology. Functionally available iron is normally found in hemoglobin, myoglobin, and enzymes (catalase and cytochromes). Additionally, ferritin is the physiological storage form (plasma ferritin is normally close to the total body Fe), and hemosiderin (Prussian blue positive) is iron precipitated in tissues in the form of degraded ferritin mixed with lysosomal debris.

Iron is transported in the blood stream by transferrin. Transferrin saturation is reported as a percentage; it represents the ratio of the serum iron to the total iron-binding capacity, multiplied by 100.

Dietary deficiency of iron is seen in elderly populations, children, and the poor. Increased demand for iron is seen in children and pregnant women. Additionally, iron deficiency can develop because of decreased absorption, either due to generalized malabsorption or more specifically after gastrectomy (due to decreased acid, which is needed for ferrous absorption) or when there is decreased small intestinal transit time (causing "dumping syndrome"). Iron deficiency can also be due to chronic blood loss due to gynecologic (menstrual bleeding) or gastrointestinal causes (in the United States, think carcinoma; in the rest of the world, think hookworm).

The sequence of events during iron deficiency is as follows:

- **Initially**, decreased storage iron results in decreased serum ferritin and decreased bone marrow iron on Prussian blue stains.
- The **next stage** is decreased circulating iron, which causes decreased serum iron, increased total iron binding capacity, and decreased % saturation.
- The **last stage** is formation of microcytic/hypochromic anemia, with decreased MCV, decreased MCHC, and high RDW.

Other clinical features of iron deficiency include increased free erythrocyte protoporphyrin (FEP), oral epithelial atrophy if Plummer-Vinson syndrome is present, koilonychia (concave or spoon nails with abnormal ridging and splitting), and pica (eating nonfood substances, e.g. dirt).

Table 11-1. Iron Panel for Microcytic Anemias

	Iron Deficiency	AOCD	Thalassemia Minor
Serum iron	↓	↓	Normal
TIBC	↑	↓	Normal
% saturation	↓	↓	Normal
Serum ferritin	↓	↑	Normal

Anemia of chronic disease (AOCD) (or anemia of inflammation) is characterized by iron being trapped in bone marrow macrophages, leading to decreased utilization of endogenous iron stores. Laboratory studies show increased serum ferritin with decreased total iron binding capacity. Increased IL-6 increases plasma hepcidin, which is a negative regulator of iron uptake in the small intestine and of iron release from macrophages.

Thalassemia syndromes are quantitative, not qualitative, abnormalities of hemoglobin. α-thalassemia has decreased α-globin chains with relative excess β chains, while β-thalassemia has decreased β-globin chains with relative excess α chains. It is hypothesized that the thalassemia genes have been selectively preserved in the human genome because the thalassemias provide a protective advantage to carriers exposed to diseases such as malaria.

Note

Composition of hemoglobins:

- HbA (2 alpha, 2 beta)
- HbA2 (2 alpha, 2 delta)
- HbF (2 alpha, 2 gamma)
- Hb Barts (4 gamma)
- Hb H (4 beta)

α-thalassemia. There are a total of 4 α-globin chain genes, 2 from each parent. α-thalassemia is due to gene deletions in the α-globin chain genes, and the clinical manifestations depend upon the number of genes that are affected. α chains are normally expressed prenatally and postnatally; therefore, there is prenatal and postnatal disease. In normal individuals, 4 α genes (αα/αα) are present and 100% of the α chains are normal.

- In the **silent carrier state**, one deletion is present, and the total number of α genes available is 3 (– α/αα), which produce 75% of the needed α chains. Individuals with the silent carrier state are completely asymptomatic and all lab tests are normal.
- In α-**thalassemia trait**, 2 deletions are present, and the total number of available α genes is 2, which produce 50% of the needed α chains. The genotype cis (– –/αα) is seen in Asians, while the genotype trans (–α/–α) is seen in African Americans (offspring don't develop hemoglobin H disease or hydrops fetalis).
- **Hemoglobin H disease** is characterized by 3 deletions, with the number of α genes being 1 (– –/– α), which produces 25% of the normal α chains. There is increased Hb H (β4,) which forms Heinz bodies that can be seen with crystal blue stain.
- **Hydrops fetalis** has 4 deletions and is lethal in utero, because the number of α genes is 0 (– –/– –), producing 0% α chains.

β-thalassemia. There are a total of 2 β-globin chain genes. In contrast to the α-globin chain genes, the 2 β-globin chain genes are expressed postnatally only, and therefore there is only postnatal disease and not prenatal disease. The damage to the genes is mainly by point mutations, which form either some β chains (β+) or none (β0).

- β-**thalassemia minor** is seen when one of the β-globin chain genes has been damaged. The condition is asymptomatic, and characterized on laboratory studies by increased hemoglobin A2 (8%) and increased hemoglobin F (5%).
- β-**thalassemia intermedia** causes varying degrees of anemia, but no transfusions are needed.
- β-**thalassemia major** (Cooley anemia). Patients are normal at birth, and symptoms develop at about 6 months as hemoglobin F levels decline. Severe hemolytic anemia results from decreased erythrocyte life span. This severe anemia causes multiple problems:
  - Intramedullary destruction results in "ineffective erythropoiesis."
  - Hemolysis causes jaundice and an increased risk of pigment (bilirubin) gallstones.
  - Lifelong transfusions are required, which result in secondary hemochromatosis.
  - Congestive heart failure (CHF) is the most common cause of death.

Erythroid hyperplasia in the bone marrow causes "crewcut" skull x-ray and increased size of maxilla ("chipmunk face"). The peripheral blood shows microcytic/hypochromic anemia with numerous target cells and increased reticulocytes. Hemoglobin electrophoresis shows increased hemoglobin F (90%), normal or increased hemoglobin A2, and decreased hemoglobin A. Treatment is hematopoietic stem cell transplantation.

Sideroblastic anemia is a disorder in which the body has adequate iron stores, but is unable to incorporate the iron into hemoglobin. It is associated with ring sideroblasts (accumulated iron in mitochondria of erythroblasts) in bone marrow. Sideroblastic anemia may be either pyridoxine (vitamin B6) responsive or pyridoxine unresponsive; the latter is a form of myelodysplastic syndrome (refractory anemia with ring sideroblasts). The peripheral blood may show a dimorphic erythrocyte population. Laboratory studies show increased serum iron, ferritin, FEP, and % saturation of TIBC, with decreased TIBC.

NORMOCYTIC ANEMIAS

Anemias of blood loss. Acute blood loss may cause shock or death. If the patient survives, the resulting hemodilution caused by shift of water from the interstitium will lower the hematocrit. There will be a marked reticulocytosis in 5–7 days. Chronic blood loss, such as from the gastrointestinal tract or from the gynecologic system, may result in iron deficiency anemia.

Hemolytic anemias.

- In **intravascular (IV) hemolysis**, release of hemoglobin into the blood causes hemoglobinemia and hemoglobinuria; increased bilirubin from erythrocytes causes jaundice and an increased risk of pigment (bilirubin) gallstones. The hemoglobin may be oxidized to methemoglobin, which causes methemoglobinemia and methemoglobinuria. Markedly decreased (because they have been used up) hemoglobin-binding proteins in the blood, such as haptoglobin and hemopexin, are characteristic. No splenomegaly is seen.

- In **extravascular (EV) hemolysis**, splenomegaly results if the EV hemolysis occurs in the spleen and hepatomegaly results if the EV hemolysis occurs in the liver. EV hemolysis causes increased bilirubin and decreased haptoglobin, but not to the degree seen with intravascular hemolysis. In EV hemolysis, there is an absence of hemoglobinemia, hemoglobinuria, and methemoglobin formation.

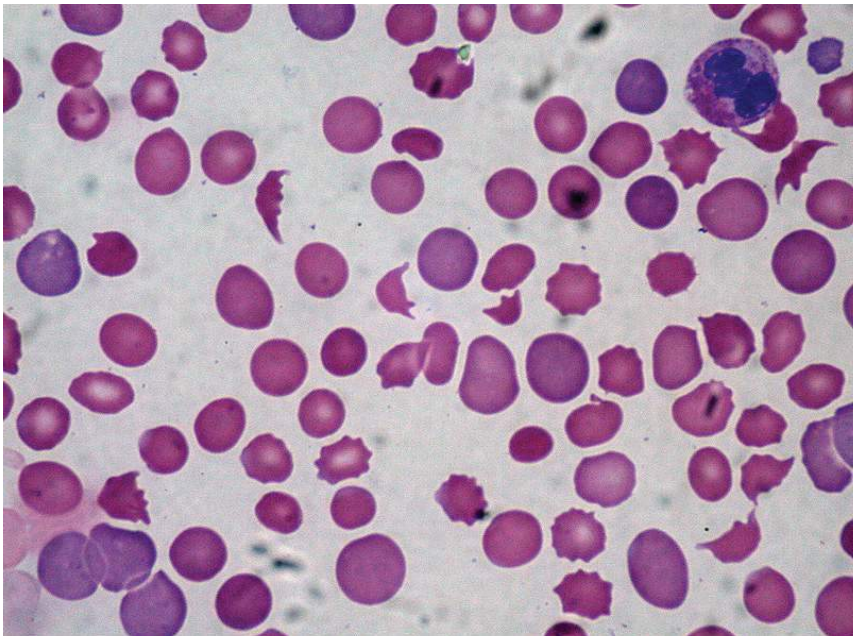

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Figure 11-2. DIC with Microangiopathic Hemolytic Anemia as Evidenced by Helmet Cells/Schistocytes

Note

Hemoglobin electrophoresis takes advantage of the differences in pH values between HbA and HbS (Glu6Val; glutamate at position 6 has been replaced by valine).

Hemoglobin electrophoresis at pH 8.4

Sickle cell disease is an inherited blood disorder leading to the formation of hemoglobin S and increased propensity for the affected red blood cells to become sickle-shaped and occlude small vessels. The genetic abnormality is a single nucleotide change that causes valine (neutral) to replace normal glutamic acid (acidic) at the sixth position of the β-globin chain. This biochemical change then makes a critical point on the surface of the hemoglobin molecule become hydrophobic, making it feel "sticky" to an adjacent hemoglobin molecule, thereby favoring hemoglobin precipitation in crystalline form.

Heterozygous (AS) genome causes sickle cell trait. About 8% of African Americans are heterozygous for hemoglobin S. Patients with sickle trait have fewer symptoms than those with sickle disease, and also have resistance to *Plasmodium falciparum* infection (malaria), which may be why the disease has remained in the human genetic pool. Homozygous (SS) genome causes clinical disease (sickle cell anemia).

There are several factors affecting formation of irreversibly sickled red blood cells.

- **Increased concentration** (dehydration) makes symptoms worse; decreased concentration of sickled hemoglobin (as is seen if a sickle cell patient also has a thalassemia) makes symptoms better.
- **Decreased pH** decreases oxygen affinity and makes symptoms worse.

- **Increased hemoglobin F** makes symptoms better (rationale for therapy with hydroxyurea, which increases blood hemoglobin F levels).
- The **presence of hemoglobin C** (SC: double-heterozygote individual) makes symptoms better.

Clinical features include increased erythrocyte destruction which causes a severe hemolytic anemia, accompanied by erythroid hyperplasia in the bone marrow and increased bilirubin leading to jaundice and gallstone (pigment) formation. Capillary thrombi result from sickle cells blocking small vessels and may cause vaso-occlusive (painful) crises; hand-foot syndrome (swelling) in children; and autosplenectomy, which is seen in older children and adults. Howell-Jolly bodies will appear in peripheral blood after autosplenectomy, and the lack of a functional spleen predisposes to increased incidence of infections (encapsulated organisms), increased incidence of *Salmonella* osteomyelitis (leg pain), leg ulcers, and risk of aplastic crisis (especially with *parvovirus B19* infection). Emergencies that may occur include priapism and acute chest syndrome.

For testing, hemoglobin electrophoresis is used to diagnose the disease, though genetic testing can be performed on amniotic fluid for prenatal diagnosis. Newborn screening is now mandatory in the United States and is commonly performed via high performance liquid chromatography. Treatment is hydroxyurea (to increase hemoglobin F) and hematopoietic stem cell transplantation.

Hemoglobin C disease occurs when a single nucleotide change in a codon causes lysine (basic) to replace normal glutamic acid (acidic) at the beta 6 position. Hemoglobin C disease is characterized by mild normochromic-normocytic anemia, splenomegaly, target cells, and rod-shaped crystals in erythrocytes (the latter being characteristic).

Glucose-6-phosphate dehydrogenase deficiency (G6PD) is a genetic disorder affecting the hexose monophosphate shunt pathway. It results in decreased levels of the antioxidant glutathione (GSH), leaving erythrocytes sensitive to injury by oxidant stresses leading to hemolysis. In some variants, G6PD is not due to decreased synthesis but rather to defective protein folding, resulting in a protein having a decreased half-life. The condition has X-linked inheritance.

- In **African Americans (A^- type)** with G6PD, the hemolysis is secondary to acute oxidative stress, such as oxidative drugs (primaquine, sulfonamides, anti-tuberculosis drugs), and more typically by viral or bacterial infections. The hemolysis is intermittent (even if the offending drug is continued) because only older erythrocytes have decreased levels of glucose-6-phosphate dehydrogenase.
- In individuals with G6PD of **Mediterranean type**, the disease is associated with favism due to ingestion of fava beans; more severe hemolysis occurs because all erythrocytes have decreased glucose-6-phosphate dehydrogenase activity in that there is both decreased synthesis and decreased stability.
- In **both forms**, the oxidation of hemoglobin forms **Heinz bodies**; these cannot be seen with normal peripheral blood stains (Wright-Giemsa) but can be visualized with supravital stains (methylene blue and crystal violet). The Heinz bodies are "eaten" by splenic macrophages (extravascular hemolysis), which may form "bite cell" erythrocytes that are visible on routine peripheral blood smears.

Bridge to Biochemistry

G6PD is the rate-limiting enzyme in the hexose-monophosphate shunt (HMP).

G6PD normally produces NADPH, which keeps glutathione reduced.

Glutathione protects RBCs by breaking down hydrogen peroxide.

Hereditary spherocytosis (HS) is an autosomal dominant disorder caused by a defect involving ankyrin and spectrin in the erythrocyte membrane; this causes a decrease in the erythrocyte surface membrane (spherocytosis). Spherocytes are not flexible and are removed in the spleen by macrophages (i.e., extravascular hemolysis). This causes multiple problems, including splenomegaly with a mild to moderate hemolytic anemia, increased bilirubin and increased risk for jaundice and pigment gallstones secondary to chronic hemolysis, and increased risk for acute red-cell aplasia due to parvovirus B19 infection. Laboratory testing shows increased osmotic fragility and normal MCH with increased MCHC. Treatment is splenectomy and folic acid.

Clinical Correlate

The differential diagnosis of spherocytes in the peripheral blood includes warm AIHA and hereditary spherocytosis. Use the osmotic fragility test (HS) and direct Coombs test (AIHA) to tell them apart. The Coombs test is negative in hereditary spherocytosis.

Autoimmune hemolytic anemia (AIHA) is most commonly warm AIHA, in which the antibodies are IgG that are usually against Rh antigens and are active at 37°C. Erythrocytes to which the antibodies attach are removed by splenic macrophages, which tends to induce splenomegaly as the spleen responds to the perceived need for increased phagocytosis.

The etiology varies; most cases are idiopathic, but some cases are related to autoimmune diseases such as systemic lupus erythematosus, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (WDLL), or medications (penicillin). The peripheral blood smear typically shows microspherocytes, and laboratory confirmation can be obtained by demonstrating a positive direct Coombs test (direct antiglobulin test [DAT]).

Paroxysmal nocturnal hemoglobinuria (PNH) is a hemolytic anemia caused by an acquired somatic mutation of a gene (*PIGA*) that encodes an anchor for proteins (CD55 and CD59) in the cell membrane, causing complement-mediated lysis in red cells, white cells, and platelets. The condition causes episodes (paroxysms) of hemolysis at night.

Clinical Correlate

Increased levels of 2,3-bisphosphoglycerate (2,3 BPG) in cells can be seen in pyruvate kinase deficiency, which may affect tissue oxygenation. This will cause a “right shift” in the oxygen-hemoglobin dissociation curve, implying hemoglobin has a decreased affinity for oxygen.

Acidosis *in vivo* occurs during sleep (breathing slowly retains CO_2) and exercise (lactic acidosis), and the acidosis in turn causes activation of complement. PNH is a clonal stem cell disorder that affects all blood cell lines, leading to pancytopenia (anemia, leukopenia, and thrombocytopenia) that is apparent in the peripheral blood. Venous thrombosis may ensue.

Most PNH patients have mild disease and don’t require treatment, but a monoclonal antibody against complement protein C5 can be therapeutic.

Pyruvate kinase deficiency is the most common enzyme deficiency in the glycolytic pathway and involves the enzyme that normally converts phosphoenolpyruvate to pyruvate. Deficiency leads to decreased ATP with resulting damage to the erythrocyte membrane. Clinically, there is a hemolytic anemia with jaundice from birth.

Hereditary elliptocytosis is a mild, hereditary, hemolytic anemia caused by a defect in spectrin. It is characterized by osmotically fragile ovoid erythrocytes (“elliptocytes”).

Aplastic anemia is the term used when marrow failure causes a pancytopenia of the blood. Idiopathic causes for aplastic anemia are most commonly seen; when the etiology is known, the aplastic anemia may be due to medications (alkylating agents, chloramphenicol), chemical agents (benzene, insecticides), infection (EBV, CMV, parvovirus, hepatitis), or whole body radiation (therapeutic or nuclear exposure).

MACROCYTIC ANEMIAS

The basic cause of **megaloblastic anemias** is impaired DNA synthesis (delayed mitoses) without impairment of RNA synthesis; this produces a nuclear-cytoplasmic asynchrony that affects all rapidly proliferating cell lines, including cells of the bone marrow, gastrointestinal tract, and gynecologic system. The erythrocytes are the most obvious rapidly proliferating cells that exhibit these changes, and specifically show megaloblastic maturation, with megaloblasts in bone marrow forming macro-ovalocytes in peripheral blood. Autohemolysis of the affected megaloblasts in bone marrow (ineffective erythropoiesis) will cause increased bilirubin and increased lactate dehydrogenase (LDH). White blood cell changes include giant metamyelocytes in bone marrow and hypersegmented neutrophils (>5 lobes) in peripheral blood. Note that platelets are not increased in size.

Megaloblastic anemia due to vitamin B_{12} (cobalamin) deficiency

- Dietary deficiency is rare because B_{12} is stored in the liver and it takes years to develop dietary deficiency; it is usually seen only in strict vegetarians (diet with no animal protein, milk, or eggs).
- Decreased absorption of vitamin B_{12} is more common and may be caused by decreased intrinsic factor associated with gastrectomy or pernicious anemia (an autoimmune gastritis); pancreatic insufficiency (pancreatic proteases normally break down B_{12}-R complexes in duodenum); or intestinal malabsorption due to parasites (fish tapeworm [*Diphyllobothrium latum*]), bacteria (blind-loop syndrome), or Crohn's disease of the ileum.
- Clinically, B_{12} deficiency causes weakness due to anemia (megaloblastic anemia) and sore ("beefy") tongue due to generalized epithelial atrophy. Unlike folate deficiency, vitamin B_{12} deficiency can also cause the central nervous system effects of subacute combined degeneration of the spinal cord, characterized by demyelination of the posterior and lateral columns of the spinal cord; the posterior (sensory) tract damage causes loss of vibratory and position sense, while the lateral cord damage involves dorsal spinocerebellar tracts (arm and leg dystaxia) and corticospinal tracts (spastic paralysis).
- Lab tests show low serum B_{12}, increased serum homocysteine, and increased methylmalonic acid in urine. Treatment is intramuscular vitamin B_{12}, which will cause increased reticulocytes in about 5 days.

Megaloblastic anemia due to folate deficiency can be caused by multiple processes:

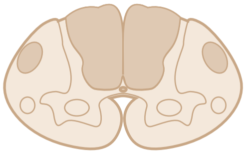
- **Decreased intake** in chronic alcoholics and the elderly
- **Decreased absorption** in the upper small intestine
- **Increased requirement for folate** during pregnancy and infancy
- Folate antagonists, e.g., methotrexate

Clinically, folate deficiency produces megaloblastic anemia without neurologic disease symptoms. Lab tests show low serum folate levels and increased serum homocysteine. Treatment is folate replacement.

Note

Normal Sequence of B_{12} Absorption

1. Dietary B12 binds to salivary haptocorrin.
2. Haptocorrin B_{12} complex is broken down by pancreatic proteases.
3. Free B_{12} binds to IF, which is secreted by gastric parietal cells.
4. B_{12}-IF complex is absorbed by ileal mucosal epithelial cells.
5. B_{12} is transported in blood bound to transcobalamin.



Subacute combined degeneration

POLYCYTHEMIA VERA

Polycythemia vera is caused by a clonal expansion of a multipotent myeloid stem cell that primarily produces extra erythrocytes. See discussion of myeloproliferative disorders in chapter 21.

Secondary polycythemia refers to increased red cell mass due to compromised ability of blood to supply oxygen to tissues. Causes include chronic obstructive pulmonary disease and cyanotic congenital heart disease. Erythropoietin levels can be appropriately high. Secondary polycythemia may also be caused by inappropriately high erythropoietin levels, with renal cell carcinoma excreting erythropoietin being the typical cause.

Relative polycythemia refers to an increased red cell count secondary to decreased plasma volume (typically due to dehydration). Red cell mass, erythropoietin, and blood oxygen content are normal.

Vascular Pathology 12

Learning Objectives

- ❑ Demonstrate understanding of the vasculitides
- ❑ Differentiate Raynaud disease from phenomenon
- ❑ Answer questions about arteriosclerosis, hypertension, aneurysms, and arteriovenous fistulas
- ❑ Explain information related to venous disease
- ❑ Demonstrate understanding of vascular neoplasms

THE VASCULITIDES

The vasculitides are a group of systemic disorders with vessel inflammation and myriad clinical presentations. There are many systems used to categorize them. The system below is based largely on the size of the vessels involved.

Large Vessel Vasculitides

Takayasu arteritis occurs in younger patients (age <50). Initial symptoms may be nonspecific (fatigue) with a variable course to more severe symptoms (blindness) and involvement of the aortic arch. Microscopically, there is vessel wall thickening and variable inflammation (from a mononuclear adventitial infiltrate to medial necrosis with granulomas).

Giant cell arteritis was formerly called temporal arteritis, but the temporal arteries are not always involved. The vertebral and ophthalmic arteries and aorta are often involved. The typical presentation evolves from nonspecific symptoms (headache) to more severe symptoms (blindness). Microscopically, there are inner media granulomas in classic cases. Treatment is steroids and anti-TNF therapy.

Note

Thromboangiitis obliterans (Buerger's disease) is often categorized with the vasculitides, but the main lesion is thrombosis; inflammation may extend from vessels into adjacent soft tissue and nerves. The disease, which presents with severe distal extremity pain and ulceration, is seen most often in young male cigarette smokers. Pharmacologic therapies have not been successful.

Medium Vessel Vasculitides

Kawasaki disease presents with mucocutaneous symptoms and cervical lymph node enlargement in children. Involvement of the coronary arteries leads to cardiovascular sequelae, which can be circumvented with immunoglobulin therapy. Microscopically, there is transmural vascular inflammation.

Polyarteritis nodosa is a systemic necrotizing vasculitis occurring most often in young adults (M > F). It has an association with hepatitis B virus. The clinical course is one of episodic nonspecific symptoms (low-grade fever). Pulmonary involvement is rare; renal artery involvement can be fatal. Immunosuppressive therapy can achieve remission in most cases.

Small Vessel Vasculitides

Small vessel vasculitides include those that are ANCA (antineutrophil cytoplasmic antibody)-associated (**granulomatosis with polyangiitis**, formerly known as Wegener's granulomatosis; and **eosinophilic granulomatosis with polyangiitis**, formerly known as Churg-Strauss syndrome) and those that are mediated by immune complexes (e.g., anti-glomerular basement membrane disease and IgA vasculitis, also known as Henoch-Schönlein purpura).

Granulomatosis with polyangiitis typically occurs in middle-aged men; it is characterized by granulomas of the lung and upper respiratory tract, glomerulonephritis, and a necrotizing granulomatous vasculitis. PR3-ANCAs are present in most cases.

Eosinophilic granulomatosis with polyangiitis is associated with asthma, extravascular granulomas (respiratory tract), and a systemic vasculitis that features eosinophils; eosinophil counts may be extremely high in peripheral blood. T lymphocytes and antibodies to MPO (P-ANCA) play a role in the etiology. There may be increased IgG4 levels. ANCA is present in cases with glomerulonephritis. The sequential phases are allergic, followed by eosinophilic and vasculitic. Cardiac involvement may be fatal. Steroids are therapeutic. Microscopic findings depend upon the organ biopsied: Purpuric leg lesions show a leukocytoclastic vasculitis; the glomerulonephritis tends not to show eosinophilic infiltrates; the extravascular pulmonary granulomas contain eosinophils.

Other small vessel vasculitides include **variable vessel vasculitides**, e.g., Behcet's disease; **single organ vasculitides**, e.g., CNS vasculitides; and **vasculitis associated with systemic disease**, e.g., rheumatoid vasculitis.

RAYNAUD DISEASE VERSUS PHENOMENON

Primary Raynaud phenomenon (Raynaud disease) typically occurs in young women as episodic small artery vasospasm in the extremities, nose, or ears; it results in blanching and cyanosis of the fingers or toes upon stress or (more commonly) exposure to cold. The pathogenesis may involve CNS and intravascular factors.

Secondary Raynaud phenomenon is caused by arterial insufficiency secondary to an underlying disease such as scleroderma (CREST).

ARTERIOSCLEROSIS

Note

Arteriosclerosis is a group of diseases that results in arterial wall thickening ("hardening of the arteries")

- Mönckeberg medial calcific sclerosis
- Arteriolosclerosis
- Atherosclerosis

Mönckeberg medial calcific sclerosis is a medial calcification of medium-sized (muscular) arteries, such as femoral, tibial, radial, and ulnar arteries. It is asymptomatic but may be detected by x-ray.

Arteriolosclerosis refers to sclerosis of arterioles; it affects small arteries and arterioles. Microscopically, either hyaline arteriolosclerosis (pink, glassy arterial wall thickening with luminal narrowing seen in benign hypertension, diabetes, and aging) or hyperplastic arteriolosclerosis (smooth-muscle proliferation resulting in concentric ["onion skin"] wall thickening and luminal narrowing seen in malignant hypertension) may occur.

Atherosclerosis is a common vascular disorder characterized by lipid deposition and intimal thickening of large and medium-sized (elastic and muscular) arteries, resulting in fatty streaks and atheromatous plaques over a period of decades

(a type of chronic inflammatory condition). Particularly likely to be affected are the aorta and a number of important muscular arteries (coronary, carotid, cerebral, renal, iliac, and popliteal arteries).

Risk factors for atherosclerosis are as follows:

Hyperlipidemia	Sedentary lifestyle
Hypertension	Stress (type A personality)
Smoking	Elevated homocysteine
Diabetes	Oral contraceptive use
Obesity	Increasing age
Male gender	Familial/genetic factors

The earliest (clinically reversible) stage in atherosclerosis is the **fatty streak**, which is seen grossly as a flat, yellow intimal streak and is characterized microscopically by lipid-laden macrophages (foam cells).

- **Stable atheromatous plaques** have a dense fibrous cap, a small lipid core and less inflammation than their vulnerable counterparts. They cause chronic ischemia.
- **Vulnerable atheromatous plaques** are at risk for rupture, thrombosis or embolization due to their composition (thin fibrous cap, large lipid core, dense inflammation).

Clinical complications of atherosclerosis are protean; these complications include ischemic heart disease (myocardial infarctions); cerebrovascular accidents (CVA); atheroemboli (transient ischemic attacks [TIAs] and renal infarcts); aneurysm formation; peripheral vascular disease; and mesenteric artery occlusion.

HYPERTENSION (HTN)

Hypertension is an elevated blood pressure leading to end-organ damage, or a sustained diastolic pressure >90 mm Hg and/or systolic pressure >140 mm Hg.

Hypertension is very common, affecting 25% of the U.S. population. African Americans tend to be more seriously affected than Caucasians, and the risk increases with age. Approximately 95% of cases of hypertension are idiopathic (essential); the remainder are due to secondary hypertension related to renal disease, pheochromocytoma, or other disease processes.

Mild to moderate elevations in blood pressure cause end-organ damage by damaging arterioles with hyaline arteriolosclerosis. Late manifestations of hypertension include concentric left ventricular hypertrophy; congestive heart failure; accelerated atherosclerosis; myocardial infarction; aneurysm formation, rupture, and dissection; intracerebral hemorrhage; and chronic renal failure.

Malignant (accelerated) hypertension accounts for 5% of the cases and is characterized by markedly elevated pressures (e.g., systolic pressure >180 mm Hg and/or diastolic >120 mm Hg), which can rapidly cause end-organ damage. Funduscopic examination may demonstrate retinal hemorrhages, exudates, and papilledema. See chapter 15 for a discussion of renal pathology. Malignant hypertension is a medical emergency; if untreated, most patients will die within 2 years from renal failure, intracerebral hemorrhage, or chronic heart failure.

ANEURYSMS AND ARTERIOVENOUS FISTULAS

Aneurysms are congenital or acquired weakness of the vessel wall media, resulting in a localized dilatation or outpouching. Complications include thrombus formation, thromboembolism, and compression of nearby structures. Rupture or dissection may cause sudden death.

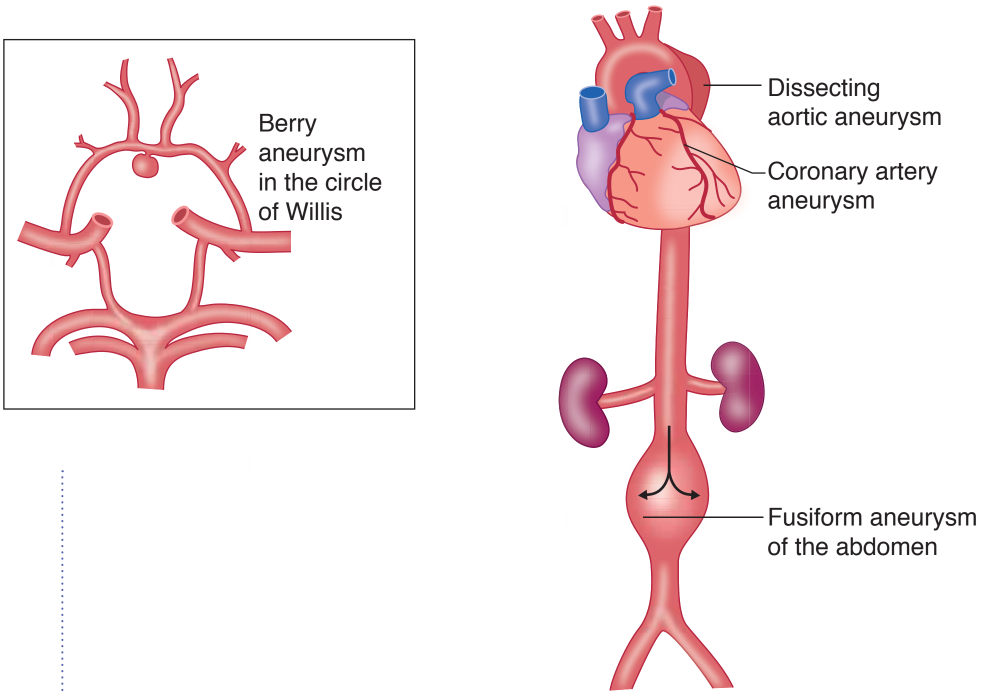


Figure 12-1. Location of Aneurysms

Atherosclerotic aneurysms are due to weakening of the media secondary to atheroma formation, and typically occur in the abdominal aorta below the renal arteries. They are associated with hypertension. Half of aortic aneurysms >6 cm in diameter will rupture within 10 years. Those >5 cm are treated surgically.

Syphilitic aneurysms involve the ascending aorta in tertiary syphilis (late stage). Syphilitic (luetic) aortitis causes an obliterative endarteritis of the vasa vasorum, leading to ischemia and smooth-muscle atrophy of the aortic media. Syphilitic aneurysms may dilate the aortic valve ring, causing aortic insufficiency.

Aortic dissecting aneurysm occurs when blood from the vessel lumen enters an intimal tear and dissects through the layers of the media. The etiology usually involves degeneration (cystic medial degeneration) of the tunica media. Aortic dissecting aneurysm presents with severe tearing pain. The dissecting aneurysm may compress and obstruct the aortic branches (e.g., renal or coronary arteries). Hypertension and Marfan syndrome are predisposing factors.

Berry aneurysm is a congenital aneurysm of the circle of Willis.

Microaneurysms are small aneurysms commonly seen in hypertension and diabetes.

Mycotic aneurysms are aneurysms usually due to bacterial or fungal infections.

Arteriovenous (AV) fistulas are a direct communication between a vein and an artery without an intervening capillary bed. They may be congenital or acquired (e.g., trauma). Potential complications include shunting of blood which may lead to high-output heart failure and risk of rupture and hemorrhage.

VENOUS DISEASE

Deep vein thrombosis (DVT) usually affects deep leg veins (90%), with iliac, femoral, and popliteal veins being particularly commonly affected. It is often asymptomatic and is consequently a commonly missed diagnosis. When symptomatic, it can produce unilateral leg swelling with warmth, erythema, and positive Homan sign (increased resistance to passive dorsiflexion of the ankle by the examiner). The diagnosis can be established with doppler "duplex" ultrasound. The major complication is pulmonary embolus.

Varicose veins are dilated, tortuous veins caused by increased intraluminal pressure. A variety of veins can be affected.

- **Superficial veins of the lower extremities** are particularly vulnerable due to a lack of structural support from superficial fat and/or incompetent valve(s). Varicosities of these superficial veins are very common (15% of the U.S. population); occur more frequently in females than males; and are common in pregnancy.
- **Esophageal varices** are due to portal hypertension (usually caused by cirrhosis) and may be a source of life-threatening hemorrhage.
- Varices of the anal region are commonly called **hemorrhoids**; are associated with constipation and pregnancy; and may be complicated by either bleeding (streaks of red blood on hard stools) or thrombosis (painful).

Venous insufficiency is more common in women than men, and the incidence increases with age. Lower extremities demonstrate edema, hyperpigmentation and ulceration due to venous hypertension and incompetent valves.

Vascular ectasias:

- **Nevus flammeus nuchae** is a neck "birthmark" or "stork bite" that regresses.
- **Port wine stain** is a vascular birthmark that does not regress.
- **Spider telangiectasias** occur on the face, blanch with pressure, and are associated with pregnancy.

Clinical Correlate

Port wine stains are large, flat, vascular malformations that are closely related to hemangiomas and are often seen on the head in the trigeminal nerve distribution.

VASCULAR NEOPLASMS

Hemangiomas are extremely common, benign vascular tumors. They are the most common tumor in infants appearing on the skin, mucous membranes, or internal organs. The major types are capillary and cavernous hemangiomas. Hemangiomas may spontaneously regress.

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Figure 12-2. Hemangioma

Hemangioblastomas are associated with von Hippel-Lindau disease, which may cause multiple hemangioblastomas involving the cerebellum, brain stem, spinal cord, and retina, as well as renal cell carcinoma.

Glomus tumors (glomangioma) are benign, small, painful tumors of the glomus body that usually occur under fingernails.

Kaposi sarcoma is a malignant tumor of endothelial cells associated with Kaposi-sarcoma–associated virus (HHV8). The condition causes multiple red-purple patches, plaques, or nodules that may remain confined to the skin or may disseminate. Microscopically, there is a proliferation of spindle-shaped endothelial cells with slit-like vascular spaces and extravasated erythrocytes.

- The **classic European form** occurs in older men of Eastern European or Mediterranean origin, who develop red-purple skin plaques on the lower extremities.
- The **transplant-associated form** occurs in patients on immunosuppression for organ transplants; involves skin and viscera; may regress with reduction of immunosuppression.
- The **African form** occurs in African children and young men in whom generalized lymphatic spread is common.
- The **AIDS-associated form** is most common in homosexual male AIDS patients; it is an aggressive form with frequent widespread visceral dissemination. Common sites of involvement include skin, GI tract, lymph nodes, and lungs. This form of Kaposi sarcoma is responsive to chemotherapy and interferon-alpha, and only rarely causes death.

Angiosarcoma (hemangiosarcoma) is a malignant vascular tumor with a high mortality that most commonly occurs in skin, breast, liver, and soft tissues. Liver angiosarcomas are associated with vinyl chloride, arsenic, and thorotrast.

Cardiac Pathology 13

Learning Objectives

- ❑ Determine the pathophysiology and different presentations of ischemic heart disease
- ❑ Define congestive heart failure and recognize the differences between left vs right heart failure
- ❑ Identify the pathophysiologic characteristics and presentation of the various valvular heart diseases
- ❑ Differentiate the pathophysiologic characteristics of the most common congenital heart diseases
- ❑ Classify the different cardiomyopathies according to clinical presentation
- ❑ Outline the clinical characteristics of carcinoid heart disease, cardiac tumors and pericardial diseases

ISCHEMIC HEART DISEASE

Cardiac ischemia is usually secondary to coronary artery disease (CAD); it is the most common cause of death in the United States. It is most often seen in middle-age men and postmenopausal women.

Angina pectoris is due to transient cardiac ischemia without cell death resulting in substernal chest pain.

- **Stable angina** (most common type) is caused by coronary artery atherosclerosis with luminal narrowing >75%. Chest pain is brought on by increased cardiac demand (exertional or emotional), and is relieved by rest or nitroglycerin (vasodilation). Electrocardiogram shows ST segment depression (subendocardial ischemia).
- **Prinzmetal variant angina** is caused by coronary artery vasospasm and produces episodic chest pain often at rest; it is relieved by nitroglycerin (vasodilatation). Electrocardiogram shows transient ST segment elevation (transmural ischemia).
- **Unstable or crescendo angina** is caused by formation of a nonocclusive thrombus in an area of coronary atherosclerosis, and is characterized by increasing frequency, intensity, and duration of episodes; episodes typically occur at rest. This form of angina has a high risk for myocardial infarction.

Myocardial infarction (MI) occurs when a localized area of cardiac muscle undergoes coagulative necrosis due to ischemia. It is the most common cause of death in the United States. The mechanism leading to infarction is coronary artery

atherosclerosis (90% of cases). Other causes include decreased circulatory volume, decreased oxygenation, decreased oxygen-carrying capacity, or increased cardiac workload, due to systemic hypertension, for instance.

- **Distribution of coronary artery thrombosis.** The left anterior descending artery (LAD) is involved in 45% of cases; the right coronary artery (RCA) is involved in 35% of cases; and the left circumflex coronary artery (LCA) is involved in 15% of cases.

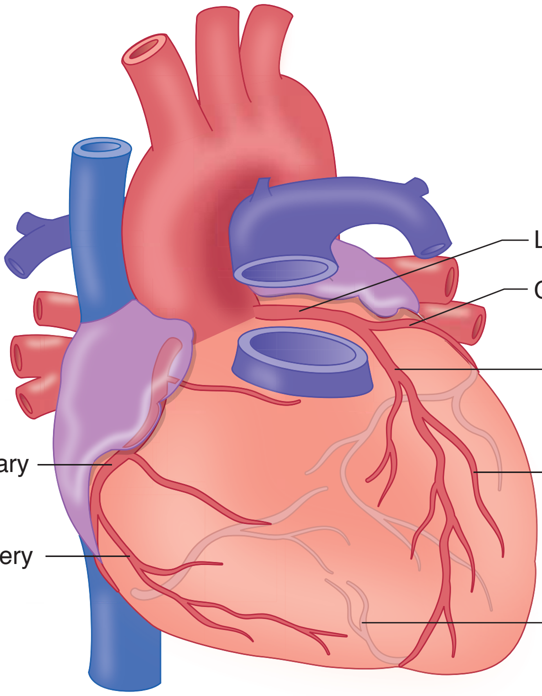


Figure 13-1. Arterial Supply to the Heart

Infarctions are classified as transmural, subendocardial, or microscopic.

- **Transmural** infarction (most common type) is considered to have occurred when ischemic necrosis involves >50% of myocardial wall. It is associated with regional vascular occlusion by thrombus. It causes ST elevated MIs (STEMIs) due to atherosclerosis and acute thrombosis.
- **Subendocardial** infarction is considered to have occurred when ischemic necrosis involves <50% of myocardial wall. It is associated with hypoperfusion due to shock. ECG changes are not noted. This type of infarction occurs in a setting of coronary artery disease with a decrease in oxygen delivery or an increase in demand.
- **Microscopic** infarction is caused by small vessel occlusion due to vasculitis, emboli, or spasm. ECG changes are not noted.

Clinical Correlate

Atypical presentation of MI with little or no chest pain is seen most frequently in elderly patients, diabetics, women, and postsurgical patients.

The clinical presentation of MI is classically a sudden onset of severe "crushing" substernal chest pain that radiates to the left arm, jaw, and neck. The pain may be accompanied by chest heaviness, tightness, and shortness of breath; diaphoresis, nausea, and vomiting; jugular venous distension (JVD); anxiety and often "feeling of impending doom." Electrocardiogram initially shows ST segment elevation. Q waves representing myocardial coagulative necrosis develop in 24–48 hours.

Table 13-1. Serum Markers Used to Diagnose Myocardial Infarctions

	Elevated by	Peak	Returns to Normal by
CK-MB	4–8 h	18 h	2–3 days
Cardiac-specific troponin I & T	3–6 h	16 h	7–10 days
LDH	24 h	3–6 days	8–14 days

The **microscopic and gross changes** represent a spectrum that is preceded by biochemical changes, going from aerobic metabolism to anaerobic metabolism within minutes. The time intervals are variable and depend on the size of the infarct, as well as other factors.

Table 13-2. Gross Sequence of Changes

Time Post-Infarction	Gross Appearance
0–12 h	No visible gross change
12–24 h	Vague pallor and softening
1–7 days	Yellow pallor
7–10 days	Central pallor with a red border
6–8 wks	White, firm scar

Table 13-3. Microscopic Sequence of Changes

Time Post-Infarction	Microscopic Appearance
1–4 h	None or wavy myocyte fibers at border or contraction band necrosis
4 h–3 days	Coagulative necrosis
1–3 days	Neutrophilic infiltrate
3–7 days	Macrophages
7–10 days	Granulation tissue
3–8 wks	Remodeled type III collagen becoming dense, collagenous scar

Complications of MI include cardiac arrhythmias that may lead to sudden cardiac death; congestive heart failure; cardiogenic shock (>40–50% myocardium is necrotic); mural thrombus and thromboembolism; fibrinous pericarditis; ventricular aneurysm; and cardiac rupture. Cardiac rupture most commonly occurs 3–7 days after MI. Effects vary with the site of rupture: ventricular free wall rupture causes cardiac tamponade; interventricular septum rupture causes left to right shunt; and papillary muscle rupture causes mitral insufficiency.

Clinical Correlate

Auscultation of a friction rub is characteristic of pericarditis. Pericarditis is most common 2–3 days after infarction but may also occur several weeks later (Dressler syndrome—a rare autoimmune reaction (type II) where the necrotic heart muscle induces the immune system to generate autoantibodies to cardiac self-antigens).

Clinical Correlate

Clinically, the degree of orthopnea is often quantified in terms of the number of pillows the patient needs in order to sleep comfortably (e.g., "three-pillow orthopnea").

Note

Cor pulmonale = right-sided heart failure caused by pulmonary hypertension from intrinsic lung disease:

- Lung disease → pulmonary HTN →↑ right ventricular pressure → right ventricular hypertrophy → right-sided heart failure.

Sudden cardiac death is defined to be death within 1 hour of the onset of symptoms. The mechanism is typically a fatal cardiac arrhythmia (usually ventricular fibrillation).

Coronary artery disease is the most common underlying cause (80%); other causes include hypertrophic cardiomyopathy, mitral valve prolapse, aortic valve stenosis, congenital heart abnormalities, and myocarditis.

Chronic ischemic heart disease is the insidious onset of progressive congestive heart failure. It is characterized by left ventricular dilation due to accumulated ischemic myocardial damage (replacement fibrosis) and functional loss of hypertrophied noninfarcted cardiac myocytes.

CONGESTIVE HEART FAILURE

Congestive heart failure (CHF) refers to the presence of insufficient cardiac output to meet the metabolic demand of the body's tissues and organs. It is the final common pathway for many cardiac diseases and has an increasing incidence in the United States. Complications include both **forward failure** (decreased organ perfusion) and **backward failure** (passive congestion of organs). Right- and left-sided heart failure often occur together.

- **Left heart failure** can be caused by ischemic heart disease, systemic hypertension, myocardial diseases, and aortic or mitral valve disease. The heart has increased heart weight and shows left ventricular hypertrophy and dilatation. The lungs are heavy and edematous. Left heart failure presents with dyspnea, orthopnea, paroxysmal nocturnal dyspnea, rales, and S3 gallop.

  Microscopically, the heart shows cardiac myocyte hypertrophy with "enlarged pleiotropic nuclei," while the lung shows pulmonary capillary congestion and alveolar edema with intra-alveolar hemosiderin-laden macrophages ("heart failure cells"). Complications include passive pulmonary congestion and edema, activation of the renin-angiotensin-aldosterone system leading to secondary hyperaldosteronism, and cardiogenic shock.

- **Right heart failure** is most commonly caused by left-sided heart failure, with other causes including pulmonary or tricuspid valve disease and *cor pulmonale*. Right heart failure presents with JVD, hepatosplenomegaly, dependent edema, ascites, weight gain, and pleural and pericardial effusions. Grossly, right ventricular hypertrophy and dilatation develop. Chronic passive congestion of the liver may develop and may progress to cardiac sclerosis/cirrhosis (only with long-standing congestion).

VALVULAR HEART DISEASE

Degenerative calcific aortic valve stenosis is a common valvular abnormality characterized by age-related dystrophic calcification, degeneration, and stenosis of the aortic valve. It is common in congenital bicuspid aortic valves. It can lead to concentric left ventricular hypertrophy (LVH) and congestive heart failure with increased risk of sudden death. The calcifications are on the outflow side of the cusps. Treatment is aortic valve replacement.

Mitral valve prolapse has enlarged, floppy mitral valve leaflets that prolapse into the left atrium and microscopically show myxomatous degeneration. The condition affects individuals with Marfan syndrome. Patients are asymptomatic and a mid-systolic click can be heard on auscultation. Complications include infectious endocarditis and septic emboli, rupture of chordae tendineae with resulting mitral insufficiency, and rarely sudden death.

Rheumatic valvular heart disease/acute rheumatic fever

Rheumatic fever is a systemic recurrent inflammatory disease, triggered by a pharyngeal infection with Group A β-*hemolytic streptococci.* In genetically susceptible individuals, the infection results in production of antibodies that cross-react with cardiac antigens (type II hypersensitivity reaction). Rheumatic fever affects children (ages 5–15 years), and there is a decreasing incidence in the United States. Symptoms occur 2–3 weeks after a pharyngeal infection; laboratory studies show elevated antistreptolysin O (ASO) titers.

The Jones criteria are illustrated below. Diagnosis of rheumatic fever requires **2 major** OR **1 major and 2 minor criteria,** plus a preceding group A strep infection.

Table 13-4. WHO Criteria for Rheumatic Fever Based on Revised Jones Criteria

Major Criteria	Minor Criteria
Carditis	Fever
Polyarthritis	Polyarthralgia
Chorea	Labs: elevated ESR or leukocyte count
Erythema marginatum	ECG: prolonged P-R interval
Subcutaneous nodules	

- **Acute rheumatic heart disease** affects myocardium, endocardium, and pericardium. The myocardium can develop myocarditis, whose most distinctive feature is the Aschoff body, in which fibrinoid necrosis is surrounded by macrophages (Anitschkow cells), lymphocytes, and plasma cells. Fibrinous pericarditis may be present. Endocarditis may be a prominent feature that typically involves mitral and aortic valves (forming fibrin vegetations along the lines of closure) and may also cause left atrial endocardial thickening (MacCallum plaques).
- **Chronic rheumatic heart disease** is characterized by mitral and aortic valvular fibrosis, characterized by valve thickening and calcification; fusion of the valve commissures; and damaged chordae tendineae (short, thickened, and fused). Complications can include mitral stenosis and/or regurgitation, aortic stenosis and/or regurgitation, congestive heart failure, and infective endocarditis.

Clinical Correlate

Endocarditis involving the tricuspid valve is highly suggestive of IV drug use or central line bacteremia.

Infectious bacterial endocarditis refers to bacterial infection of the cardiac valves, characterized by vegetations on the valve leaflets. Risk factors include rheumatic heart disease, mitral valve prolapse, bicuspid aortic valve, degenerative calcific aortic stenosis, congenital heart disease, artificial valves, indwelling catheters, dental procedures, immunosuppression, and intravenous drug use.

- **Acute endocarditis** is typically due to a *high virulence organism* that can colonize a normal valve, such as *Staphylococcus aureus*. Acute endocarditis produces large destructive vegetations (fibrin, platelets, bacteria, and neutrophils). The prognosis is poor, with mortality of 10–40%.
- **Subacute endocarditis** is typically due to a low virulence organism, such as *Streptococcus group viridans*, which usually colonizes a previously damaged valve. The disease course is typically indolent with <10% mortality.

Bridge to Microbiology

Viridans streptococci

- Alpha-hemolytic
- Bile-resistant
- Optochin-resistant

Clinically, endocarditis presents with fever, chills, weight loss, and cardiac murmur. Embolic phenomena may occur, and may affect systemic organs; retina (Roth spots); and distal extremities (Osler nodes [painful, red subcutaneous nodules on the fingers and toes], Janeway lesions [painless, red lesions on the palms and soles], and splinter fingernail hemorrhages). Diagnosis is by serial blood cultures. Complications include septic emboli, valve damage resulting in insufficiency and congestive heart failure, myocardial abscess, and dehiscence of an artificial heart valve.

Clinical Correlate

S. bovis endocarditis or septicemia is associated with a higher incidence of occult colorectal tumors.

Colonoscopy should be performed in all patients with *S. bovis* bacteremia or endocarditis.

Marantic endocarditis (nonbacterial thrombotic endocarditis [NBTE]) is characterized by small, sterile vegetations along the valve leaflet line of closure in patients with a debilitating disease. The major complications are embolism and secondary infection of the vegetations.

MYOCARDITIS

Myocarditis is caused by infectious (Coxsackie A and B viruses, Chagas disease) and immune causes. Clinically, the patient may be asymptomatic or may suffer from acute heart failure or even dilated cardiomyopathy.

CONGENITAL HEART DISEASE

Congenital heart disease is the most common cause of childhood heart disease in the United States; 90% of cases are idiopathic and 5% are associated with genetic disease (trisomies, cri du chat, Turner syndrome, etc.), viral infection (especially congenital rubella), or drugs and alcohol.

Coarctation of the aorta is a segmental narrowing of the aorta.

- **Preductal coarctation** (infantile-type) is associated with Turner syndrome and causes severe narrowing of aorta proximal to the ductus arteriosus. It is usually associated with a patent ductus arteriosus (PDA), which supplies blood to aorta distal to the narrowing, and right ventricular hypertrophy (secondary to the need for the right ventricle to supply the aorta through the patent ductus arteriosus). It presents in infancy with congestive heart failure that is accompanied by weak pulses and cyanosis in the lower extremities; the prognosis is poor without surgical correction.

- **Postductal coarctation** (adult-type) causes stricture or narrowing of the aorta distal to the ductus arteriosus. It can present in a child or an adult with hypertension in the upper extremities, and hypotension and weak pulses in the lower extremities. Some collateral circulation may be supplied via the internal mammary and intercostal arteries; the effects of this collateral circulation may be visible on chest x-ray with notching of the ribs due to bone remodeling as a consequence of increased blood flow through the intercostal arteries.

  Complications can include congestive heart failure (the heart is trying too hard), intracerebral hemorrhage (the blood pressure in the carotid arteries is too high), and dissecting aortic aneurysm (the blood pressure in the aortic route is too high).

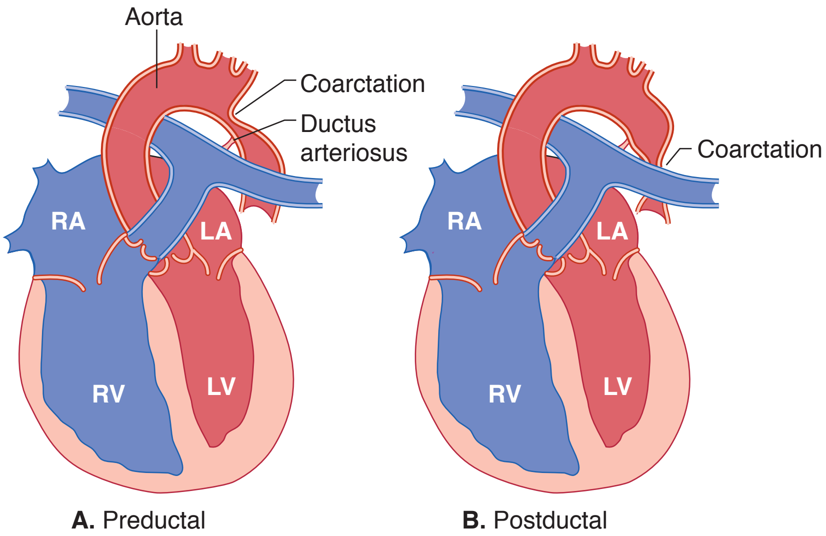


Figure 13-2. Coarctation of the Aorta

Table 13-5. Left Versus Right Shunt Congenital Disease

Right → Left Shunt	Left → Right Shunt
Early cyanosis (blue babies)	Late cyanosis (blue kids)
Blood shunted past the lungs	Secondary pulmonary HTN → reversal of shunt (Eisenmenger syndrome)
• Tetralogy of Fallot • Transposition of the great vessels • Truncus arteriosus • Tricuspid atresia	• Ventricular septal defect • Atrial septal defect • Patent ductus arteriosus

Note

"Overriding aorta" refers to the location of the aorta relative to the VSD.

Tetralogy of Fallot is the most common cause of congenital cyanotic heart disease. The classic tetrad includes **right ventricular outflow obstruction/stenosis; right ventricular hypertrophy; ventricular septal defect;** and **overriding aorta**. Clinical findings include cyanosis, shortness of breath, digital clubbing, and polycythemia. Progressive pulmonary outflow stenosis and cyanosis develop over time; treatment is surgical correction.

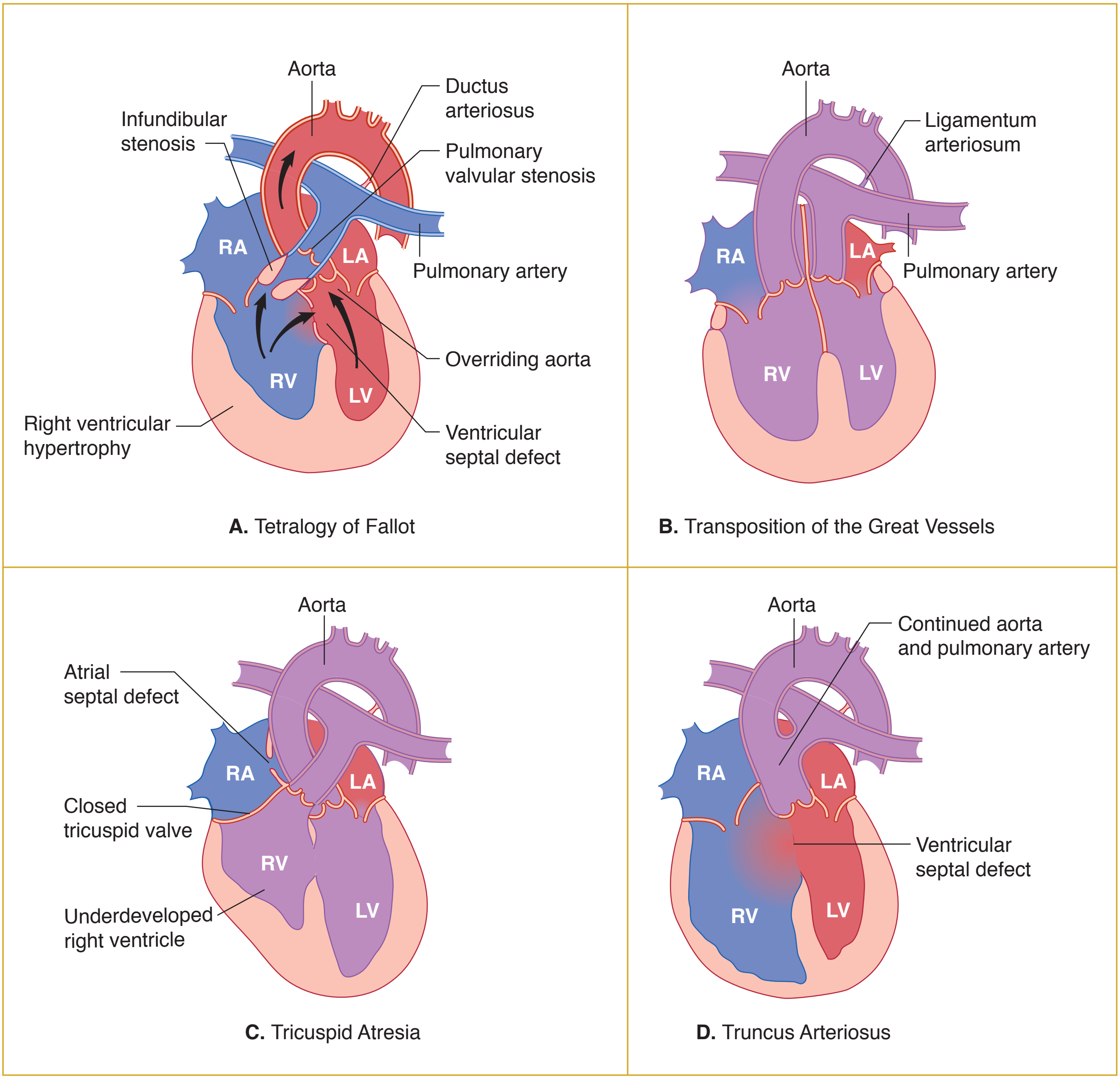


Figure 13-3. Common Forms of Cyanotic Congenital Heart Disease

Transposition of the great vessels is an abnormal development of the truncoconal septum whereby the aorta arises from the right ventricle, and the pulmonary artery arises from the left ventricle. The risk is increased in infants of diabetic mothers. Affected babies develop early cyanosis and right ventricular hypertrophy. To survive, infants must have mixing of blood by a VSD, ASD, or PDA. The prognosis is poor without surgery.

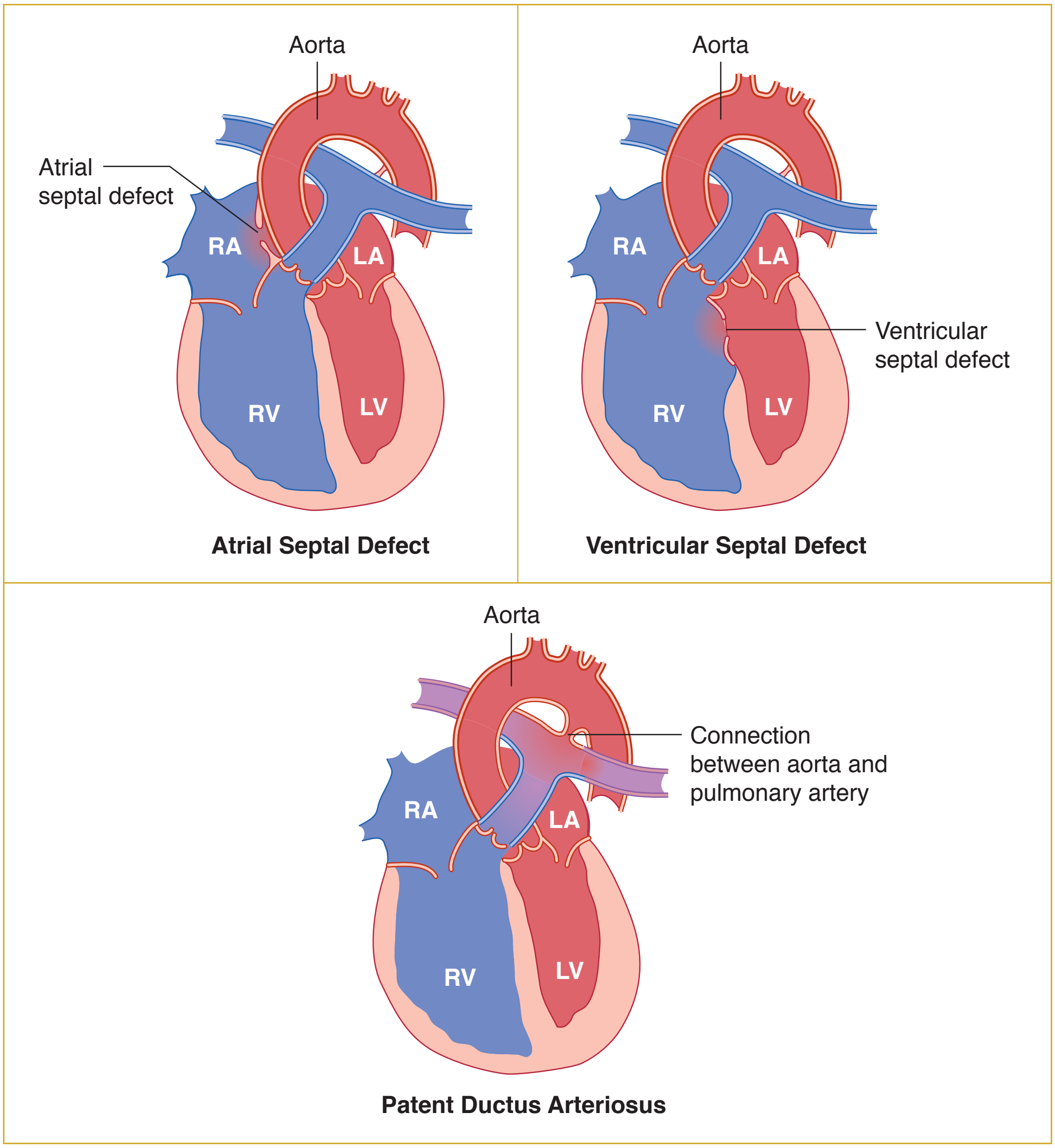


Figure 13-4. Common Forms of Acyanotic Congenital Heart Disease

Truncus arteriosus is a failure to develop a dividing septum between the aorta and pulmonary artery, resulting in a common trunk. Blood flows from the pulmonary trunk to the aorta. Truncus arteriosus causes early cyanosis and congestive heart failure, with a poor prognosis without surgery.

Tricuspid atresia refers to the absence of a communication between the right atrium and ventricle due to developmental failure to form the tricuspid valve. Associated defects include right ventricular hypoplasia and an ASD. The prognosis is poor without surgery.

Bridge to Pharmacology

To keep PDA open: prostaglandin E2

To close PDA: indomethacin

Bridge to Embryology

In utero the ductus arteriosus is kept open by low arterial oxygen saturation and elevated prostaglandin E2 (PGE2) levels. Functional closure occurs in the first 2 days of life due to increased oxygen saturation and decreased PGE2. The ductus arteriosus becomes the ligamentum arteriosum.

Ventricular septal defect (VSD), which consists of a direct communication between the ventricular chambers, is the second most common congenital heart defect (the most common is a bicuspid aortic valve).

- A **small ventricular septal defect** may be asymptomatic and close spontaneously, or it may produce a jet stream that damages the endocardium and increases the risk of infective endocarditis.
- A **large ventricular septal defect** may cause Eisenmenger complex, which is characterized by secondary pulmonary hypertension, right ventricular hypertrophy, reversal of the shunt, and late cyanosis.
- In **both types**, a systolic murmur can be heard on auscultation. Ventricular septal defects are commonly associated with other heart defects. Large ventricular septal defects can be surgically corrected.

Atrial septal defect (ASD) is a direct communication between the atrial chambers. The most common type is an ostium secundum defect. Complications include Eisenmenger syndrome and paradoxical emboli.

Patent ductus arteriosus (PDA) is a direct communication between the aorta and pulmonary artery due to the continued patency of the ductus arteriosus after birth. It is associated with prematurity and congenital rubella infections. Clinical findings include machinery murmur, late cyanosis, and congestive heart failure. Eisenmenger syndrome may develop as a complication.

PRIMARY CARDIOMYOPATHIES (DIAGNOSIS OF EXCLUSION)

Dilated cardiomyopathy (most common form) is cardiac enlargement with dilatation of all 4 chambers, resulting in progressive congestive heart failure (typical mode of presentation). The cause is genetic in 20–50% of cases, but some cases are related to alcohol, medications (Adriamycin [doxorubicin]), cocaine, viral myocarditis (Coxsackievirus B and enteroviruses), parasitic infections (Chagas disease), iron overload or pregnancy.

In cases of all types, the underlying etiology leads to destruction of myocardial contractility, which affects systolic function. Echocardiogram typically shows decreased ejection fraction. Complications include mural thrombi and cardiac arrhythmias; prognosis is poor with 5-year survival of 25%. Treatment is heart transplantation. There is myocyte hypertrophy with interstitial fibrosis on microscopy, and eccentric hypertrophy seen on gross examination.

Hypertrophic cardiomyopathy (also called asymmetrical septal hypertrophy, and idiopathic hypertrophic subaortic stenosis [IHSS]) is a common cause of sudden cardiac death in young athletes. The condition is an asymmetrical hypertrophy of cardiac muscle that causes decreased compliance affecting diastolic function. Hypertrophic cardiomyopathy can be an autosomal dominant disorder (>50% of cases) or idiopathic. The muscle hypertrophy is due to the increased synthesis of actin and myosin, and on microscopic examination, the cardiac muscle fibers are hypertrophied and in disarray. Hypertrophic cardiomyopathy is most prominent in the ventricular septum, where it can obstruct the ventricular outflow tract. This can potentially lead to death during severe exercise when the cardiac outflow tract collapses, preventing blood from exiting the heart.

Restrictive cardiomyopathy (uncommon form) is caused by diseases which produce restriction of cardiac filling during diastole; etiologies include amyloidosis, sarcoidosis, endomyocardial fibroelastosis, and Loeffler endomyocarditis. In all of these diseases, increased deposition of material leads to decreased compliance, affecting diastolic function. On gross examination, the ventricles are not enlarged and the cavities are not dilated. Microscopy will reflect the underlying cause.

Arrhythmogenic right ventricular cardiomyopathy causes thinning of the right ventricle due to autosomal dominant mutations that encode desmosomal junctional proteins. On microscopy, there is fatty infiltration of the myocardium.

CARCINOID HEART DISEASE

Carcinoid heart disease is right-sided endocardial and valvular fibrosis secondary to serotonin exposure in patients with carcinoid tumors that have metastasized to the liver. It is a plaque-like thickening (endocardial fibrosis) of the endocardium and valves of the right side of the heart. Many patients experience carcinoid syndrome (also related to secretion of serotonin and other metabolically active products of the tumors), characterized by skin flushing, diarrhea, cramping, bronchospasm, wheezing, and telangiectasias. The diagnosis can be established by demonstrating elevated urinary 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of the breakdown of serotonin via monoamine oxidase.

CARDIAC TUMORS

Primary cardiac tumors are rare. The majority are benign; the malignant tumors are sarcomas. Treatment is excision.

- **Cardiac myxoma** is a benign tumor usually arising within the left atrium near the fossa ovalis in decades 3-6 of life; it can present like mitral valve disease. In 10% of cases there is an autosomal dominant condition known as Carney complex (myxomas with endocrine abnormalities and lentigines or pigmented nevi). Cardiac myxoma is characterized microscopically by stellate-shaped cells within a myxoid background. Complications include tumor emboli and “ball-valve” obstruction of the valves.
- **Cardiac rhabdomyoma** is a benign tumor usually arising within the myocardium that is associated with tuberous sclerosis.

Note

Tuberous Sclerosis Complex

- Autosomal dominant
- Mutation in genes *TSC1* and *TSC2*, which encode tumor suppressor proteins hamartin and tuberin, respectively
- Multiple hamartomas
- Cortical tubers
- Renal angiomyolipomas
- Cardiac rhabdomyomas
- Pulmonary hamartomas

PERICARDIAL DISEASE

Pericarditis. There are 2 kinds of pericarditis, acute and chronic.

- **Acute pericarditis** is characterized by a fibrinous exudate (viral infection or uremia) or by a fibrinopurulent exudate (bacterial infection).
- **Chronic pericarditis** can occur when acute pericarditis does not resolve and adhesions form.

Pericardial effusion may be serous (secondary to heart failure or hypoalbuminemia), serosanguineous (due to trauma, malignancy, or rupture of the heart or aorta) or chylous (due to thoracic duct obstruction or injury).

Tumors of the lung and breast may spread by **direct extension** to the pericardia.

Respiratory Pathology 14

Learning Objectives

- ❑ Explain information related to congenital cystic lung lesions
- ❑ Demonstrate understanding of atelectasis
- ❑ Solve problems concerning pulmonary infections
- ❑ Demonstrate understanding of sarcoidosis
- ❑ Answer questions about obstructive versus restrictive lung disease
- ❑ Answer questions about vascular disorders of the lungs
- ❑ Demonstrate understanding of pulmonary and laryngeal neoplasias
- ❑ Explain information related to diseases of the pleural cavity

CONGENITAL CYSTIC LUNG LESIONS

The 2 most common malformations of the lung are **congenital cystic adenomatoid malformation** (CCAM) and **bronchopulmonary sequestration** (BPS). CCAM is a hamartomatous lesion, and BPS is a nonfunctioning bronchopulmonary segment separate from the tracheobronchial tree.

These conditions are followed by serial ultrasonography. Some resolve spontaneously, though minimally invasive surgery may be required.

ATELECTASIS

Atelectasis refers to an area of collapsed or nonexpanded lung. It is reversible, but areas of atelectasis predispose for infection due to decreased mucociliary clearance.

The major types are as follows:

- **Obstruction/resorption atelectasis** is collapse of lung due to resorption of air distal to an obstruction; examples include aspiration of a foreign body, chronic obstructive pulmonary disease (COPD), and postoperative atelectasis.
- **Compression atelectasis** is atelectasis due to fluid, air, blood, or tumor in the pleural space.
- **Contraction (scar) atelectasis** is due to fibrosis and scarring of the lung.
- **Patchy atelectasis** is due to a lack of surfactant, as occurs in hyaline membrane disease of newborn or acute (adult) respiratory distress syndrome (ARDS).

Bridge to Anatomy

Pores of Kohn are collateral connections between alveoli through which infections and neoplastic cells can spread.

PULMONARY INFECTIONS

In **bacterial pneumonia,** acute inflammation and consolidation (solidification) of the lung are due to a bacterial agent. Clinical signs and symptoms include fever and chills; productive cough with yellow-green (pus) or rusty (bloody) sputum; tachypnea; pleuritic chest pain; and decreased breath sounds, rales, and dullness to percussion.

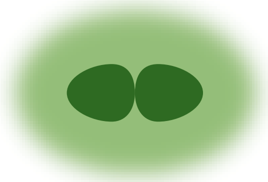
Studies typically show elevated white blood cell count with a left shift (an increase in immature leukocytes). Chest x-ray for lobar pneumonia typically shows lobar or segmental consolidation (opacification), and for bronchopneumonia typically shows patchy opacification. Pleural effusion may also be picked up on chest x-ray.

In general, the keys to effective therapy are identification of the organism and early treatment with antibiotics.

Lobar pneumonia is characterized by consolidation of an entire lobe. The infecting organism is typically *Streptococcus pneumoniae* (95%) or *Klebsiella*. The lancet-shaped diplococcus *Streptococcus pneumoniae* is alpha-hemolytic, bile soluble, and optochin sensitive.

The **4 classic phases** of lobar pneumonia are **congestion** (active hyperemia and edema); **red hepatization** (neutrophils and hemorrhage); **gray hepatization** (degradation of red blood cells); and **resolution** (healing). In today's antibiotic era, these changes are not generally observed in practice.

Bronchopneumonia is characterized by scattered patchy consolidation centered on bronchioles; the inflammation tends to be bilateral, multilobar, and basilar, and particularly susceptible populations include the young, old, and terminally ill. Infecting organisms exhibit more variation than in lobar pneumonia, and include *Staphylococci, Streptococci, Haemophilus influenzae, Pseudomonas aeruginosa,* etc. Microscopic examination of tissue shows acute inflammation of bronchioles and surrounding alveoli. The diagnosis can often be established with sputum Gram stain and sputum culture, but will sometimes require blood cultures.



Streptococcus pneumoniae

Complications of pneumonia include fibrous scarring and pleural adhesions, lung abscess, empyema (pus in a body cavity), and sepsis.

Treatment of pneumonia is generally initial empiric antibiotic treatment, modified by the results of cultures and organism sensitivities.

Lung abscess is a localized collection of neutrophils (pus) and necrotic pulmonary parenchyma. The etiology varies with the clinical setting. **Aspiration** is the most common cause. It tends to involve right lower lobe and typically has mixed oral flora (often both anaerobic and aerobic) for infecting organisms.

Lung abscess may also occur following a pneumonia, especially one due to *S. aureus* or *Klebsiella*. Lung abscesses may also occur following airway obstruction (postobstructive) or deposition of septic emboli in the lung.

Complications of lung abscess include empyema, pulmonary hemorrhage, and secondary amyloidosis.

Atypical pneumonia is the term used for interstitial pneumonitis without consolidation. It is more common in children and young adults.

Infecting organisms that can cause atypical pneumonia include *Mycoplasma pneumoniae, influenza virus, parainfluenza virus, respiratory syncytial virus* (RSV) (which is especially important in young children), *adenovirus, cytomegalovirus* (CMV) (which is especially important in the immunocompromised), *varicella virus*, and many others.

Diagnosis. Chest x-ray typically shows diffuse interstitial infiltrates. An elevated cold agglutinin titer specifically suggests *Mycoplasma* as a cause, which is important to identify since antibiotic therapy for *Mycoplasma* exists. Lung biopsy, if performed, typically shows lymphoplasmacytic inflammation within the alveolar septa.

Complications include superimposed bacterial infections and Reye syndrome (potentially triggered by viral illness [influenza/varicella] treated with aspirin).

Tuberculosis (TB). The number of cases of TB is declining in the United States, but the proportion of cases in people born outside the country is rising. In this clinical setting, a positive PPD skin test may demonstrate that the person has been exposed to the mycobacterial antigens. Individuals who have received the BCG vaccine in some foreign countries may have a positive PPD test without being infected. In such cases chest x-ray and sputum smears and cultures are done.

Note

BCG (Bacillus Calmette-Guérin) is a tuberculosis vaccine prepared from a strain of attenuated live bovine tuberculosis bacillus, *Mycobacterium bovis*.

Infection is usually acquired by inhalation of aerosolized bacilli.

The **clinical presentation** of *Mycobacterium tuberculosis* includes fevers and night sweats, weight loss, cough, and hemoptysis.

Primary pulmonary TB develops on initial exposure to the disease. The Ghon focus of primary TB is characterized by subpleural caseous granuloma formation, either above or below the interlobar fissure. The term *Ghon complex* refers to the combination of the Ghon focus and secondarily-involved hilar lymph nodes with granulomas. Most primary pulmonary tuberculosis lesions (95%) will undergo fibrosis and calcification.

Progressive pulmonary TB can take several forms, including cavitary tuberculosis, miliary pulmonary tuberculosis, and tuberculous bronchopneumonia.

Secondary pulmonary TB (also known as postprimary or reactivation TB) occurs either with reactivation of an old, previously quiescent infection or with reinfection secondary to a second exposure to the mycobacteria. In secondary pulmonary TB, the infection often produces a friable nodule at the lung apex (Simon focus) secondary to the high oxygen concentration present at that site, since the upper parts of the lung typically ventilate more efficiently than the lower parts. Biopsy of affected tissues will typically show AFB-positive caseating granulomas.

Additionally, **dissemination to other organ systems** can occur in advanced TB via a hematogenous route that often results in a miliary pattern within each affected organ. Sites that may become involved include meninges; cervical lymph nodes (scrofula) and larynx; liver/spleen, kidneys, adrenals, and ileum; lumbar vertebrae bone marrow (Pott disease); and fallopian tubes and epididymis.

Nontuberculous mycobacteria. *M. avium* complex (MAC) typically occurs in AIDS patients with CD4 counts <50 cells/mm^3 and presents as disseminated disease.

The diagnosis of TB requires identification of the bacilli. Positive sputum smear necessitates culture for species identification. Adequate treatment requires drug susceptibility testing.

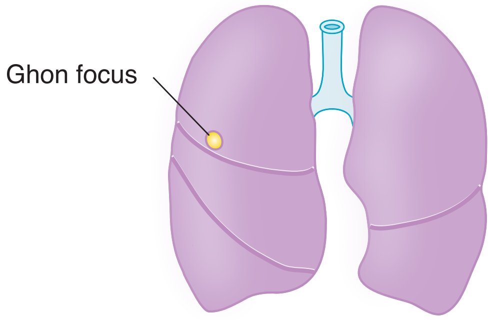


Figure 14-1. Primary Tuberculosis

SARCOIDOSIS

Sarcoidosis is a systemic granulomatous disease of uncertain etiology. The disease affects females more than males, with typical age 20–60. It is most common in African American women. Clinical presentation varies. It may be asymptomatic, or presenting symptoms may include cough and shortness of breath; fatigue and malaise; skin lesions; eye irritation or pain; and fever or night sweats. Most often, the disease is first detected on chest x-ray as bilateral hilar lymphadenopathy or parenchymal infiltrates.

The noncaseating granulomas that are characteristic of sarcoidosis may occur in **any organ of the body**. In the lung, they typically form diffuse scattered granulomas; lymph node involvement may cause hilar and mediastinal adenopathy. Skin, liver and/or spleen, heart, central nervous system, bone marrow, and gastrointestinal tract are also frequent targets of the disease. Eye involvement can be seen in Mikulicz syndrome (involvement of the uvea and parotid).

The diagnosis of sarcoidosis can be suggested by clinical studies. In the laboratory, serum angiotensin converting enzyme (ACE), which is synthesized by endothelial cells and macrophages, may be elevated. X-ray studies frequently show bilateral hilar lymphadenopathy.

Sarcoidosis is considered to be a **diagnosis of exclusion**. In practice, this means that the diagnosis is considered when a biopsy shows features characteristic of sarcoidosis (such as noncaseating granulomas, Schaumann bodies [laminated dystrophic calcification], and asteroid bodies [stellate giant cell cytoplasmic inclusions]). There are no pathognomonic microscopic features though, and the diagnosis requires clinicopathologic correlation.

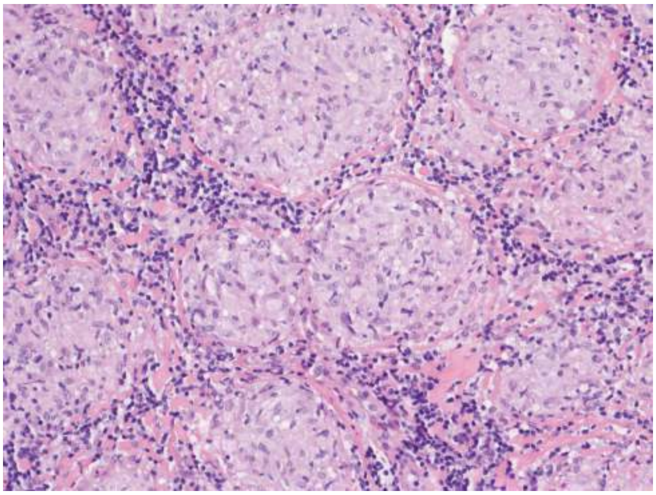


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Figure 14-2. Noncaseating Sarcoid Granulomas

The prognosis is favorable with a variable clinical course. Most patients completely recover but some succumb to respiratory compromise.

OBSTRUCTIVE VERSUS RESTRICTIVE LUNG DISEASE

Table 14-1. Obstructive Versus Restrictive Lung Disease

Obstructive Airway Disease	Restrictive Lung Disease
Definition: Increased resistance to airflow secondary to obstruction of airways	Decreased lung volume and capacity
Pulmonary function tests (spirometry) FEV_1/FVC ratio is decreased	Decreased TLC and VC
Examples: Chronic obstructive airway disease • Asthma • Chronic bronchitis • Emphysema • Bronchiectasis	Chest wall disorders • Obesity, kyphoscoliosis, polio, etc. Interstitial/infiltrative diseases • ARDS, pneumoconiosis • Pulmonary fibrosis

Table 14-2. Summary of Obstructive Versus Restrictive Pattern

Variable	Obstructive Pattern, e.g., Emphysema	Restrictive Pattern, e.g., Fibrosis
Total lung capacity	increased	decreased
FEV_1	decreased	decreased
Forced vital capacity	normal or slightly decreased	decreased
FEV_1/FVC	decreased	increased or normal
Peak flow	decreased	decreased
Functional residual capacity	increased	decreased
Residual volume	increased	decreased

OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a general term used to indicate chronic decreased respiratory function due to chronic bronchitis or emphysema. Both diseases are associated with smoking.

Chronic bronchitis is a clinical diagnosis made when a patient has a persistent cough and copious sputum production for at least 3 months in 2 consecutive years. It is highly associated with smoking (90%). Clinical findings include cough, sputum production, dyspnea, frequent infections, hypoxia, cyanosis, and weight gain.

Microscopic examination demonstrates hypertrophy and hyperplasia of bronchial mucous glands (Reid index equals the submucosal gland thickness divided by the bronchial wall thickness between the pseudostratified columnar epithelium and the perichondrium; normal ratio is ≤0.4).

Complications include increased risk for recurrent infections; secondary pulmonary hypertension leading to right heart failure (cor pulmonale) and lung cancer.

Emphysema is the term used when destruction of alveolar septa results in enlarged air spaces and a loss of elastic recoil. The 4 types of emphysema are named for the anatomical distribution of the septal damage.

- In **centrilobular emphysema**, the damage is in the proximal portion of the acinus and the cause is cigarette smoking.
- In **panacinar emphysema**, the damage affects the entire acinus and the common cause is alpha-1 antitrypsin deficiency.
- In **distal acinar emphysema** (unknown cause), extension to the pleura causes pneumothorax.
- In **irregular emphysema**, post-inflammatory scarring involves the acinus in an irregular distribution.

The etiology of emphysema involves a **protease/antiprotease imbalance**. On gross examination, the lungs are overinflated and enlarged, and have enlarged, grossly visible air spaces. Clinical findings include progressive dyspnea, pursing of lips and use of accessory respiratory muscles to breathe, barrel chest (increased anterior-posterior diameter), and weight loss.

Table 14-3. Manifestations Related to Area of Involvement

Centriacinar (Centrilobular)	Panacinar (Panlobular)
Proximal respiratory bronchioles involved, distal alveoli spared	Entire acinus involved
Most common type (95%)	
Associated with smoking, air pollution	α-1-antitrypsin deficiency
Distribution: worse in apical segments of upper lobes	Distribution: entire lung; worse in bases of lower lobes

Asthma is due to hyperreactive airways, which undergo episodic bronchospasm when triggered by certain stimuli.

- **Atopic (type I IgE-mediated hypersensitivity reaction) asthma** (most common form) usually affects children and young adults. There is often a positive family history.
- **Nonatopic asthma** is triggered by processes including respiratory infections (usually viral), stress, exercise, or cold temperatures.
- **Drug-induced asthma** affects about 10% of adults with a diagnosis of asthma. Aspirin is a key example of a precipitating drug.
- **Occupational asthma** is caused by workplace triggers including fumes and dusts.

An asthma attack is characterized by wheezing, severe dyspnea, and coughing. Problems with expiration cause lung overinflation. Status asthmaticus is a potentially fatal unrelenting attack of asthma.

Microscopic examination of sputum cytology may show Curschmann spirals (twisted mucus plugs admixed with sloughed epithelium), eosinophils, or Charcot-Leyden crystals (protein crystalloids from broken down eosinophils).

In patients dying from disease, autopsy findings include mucus plugs, increased mucous glands with goblet cell hyperplasia, inflammation (especially with eosinophils), edema; hypertrophy and hyperplasia of bronchial wall smooth muscle, and thickened basement membranes.

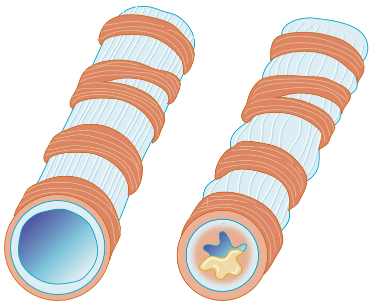

Figure 14-3. Bronchial Changes in Asthma

Bronchiectasis is an abnormal permanent airway dilatation due to chronic necrotizing inflammation. Clinical findings include cough, fever, malodorous purulent sputum, and dyspnea. Causes are diverse, and include bronchial obstruction by foreign body, mucus, or tumor, necrotizing pneumonias, cystic fibrosis, and Kartagener syndrome.

- **Kartagener syndrome** is an autosomal recessive condition caused by immotile cilia due to a defect of dynein arms (primary ciliary dyskinesia). It is characterized clinically by bronchiectasis, chronic sinusitis, and situs inversus (a congenital condition where the major visceral organs are anatomically reversed compared with their normal anatomical positions).

On gross examination, bronchiectasis shows dilated bronchi and bronchioles extending out to the pleura. These changes may also be appreciated on chest x-ray. Complications include abscess, septic emboli, cor pulmonale, and secondary amyloidosis.

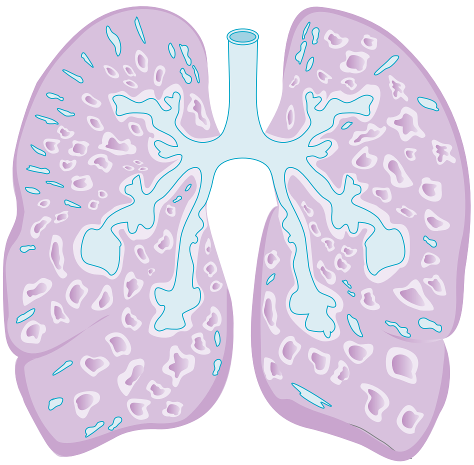

Figure 14-4. Cross-Section of Bronchiectasis

INFILTRATIVE RESTRICTIVE LUNG DISEASES (DIFFUSE INTERSTITIAL DISEASES)

Acute respiratory distress syndrome (ARDS) refers to diffuse damage of alveolar epithelium and capillaries, resulting in progressive respiratory failure that is unresponsive to oxygen treatment. Clinicians use the term *ARDS*, while pathologists use the term *diffuse alveolar damage (DAD)* to describe the pathologic changes.

ARDS may be caused by shock, sepsis, trauma, gastric aspiration, radiation, oxygen toxicity, drugs, or pulmonary infection. Activated neutrophils mediate cell damage. Clinically, patients show dyspnea, tachypnea, hypoxemia, cyanosis, and use of accessory respiratory muscles. X-rays show bilateral lung opacity ("white out").

On gross pathologic examination affected lungs are heavy, stiff, and noncompliant. Microscopically, there is intra-alveolar edema, and hyaline membranes line the alveolar spaces. In resolving cases there is proliferation of type II pneumocytes and interstitial inflammation and fibrosis.

Bridge to Anatomy

Type II pneumocytes repopulate the alveolar epithelium in response to injury.

Treatment is based on treating the underlying cause and on supporting respiration with mechanical ventilation. The prognosis is problematic even with good care, with overall mortality 40%.

Respiratory distress syndrome of the newborn (hyaline membrane disease of newborns) is caused by a deficiency of surfactant. It is associated with prematurity (gestational age of <28 weeks has a 60% incidence), maternal diabetes, multiple births, male gender, and cesarean section delivery. Clinically, infants are normal at birth but within a few hours develop increasing respiratory effort, tachypnea, nasal flaring, use of accessory muscles of respiration, an expiratory grunt, and cyanosis. Chest radiograph may demonstrate bilateral "ground-glass" reticulogranular densities. Autopsy findings include atelectasis and hyaline membranes.

Treatment is surfactant replacement, mechanical ventilation, and continuous positive airway pressure (CPAP). Respiratory distress syndrome of the newborn can sometimes be prevented if labor can be delayed and if corticosteroids are used to mature the lung. With improved therapies, now >90% of babies survive.

Chronic interstitial lung disease is a term describing heterogeneous lung disorders which share similar symptomology but vary in prognosis. Patients present with dyspnea and cough. Lung biopsy findings can be paired with clinical information to aid in therapeutic management/palliative care.

- **Idiopathic pulmonary fibrosis (IPF)** is a fatal disease. It shows patchy interstitial fibrosis and inflammation. "Honeycomb fibrosis" refers to dilated cystic spaces lined with type II pneumocytes; this histology is consistent with end-stage lung. Pathologists use the term *usual interstitial pneumonia* for IPF.
- **Nonspecific interstitial pneumonia** has a better prognosis than IPF. There is a cellular pattern and a fibrosing pattern.
- **Cryptogenic organizing pneumonia** responds to steroids. Histologically, there are plugs of connective tissue inside the alveolar spaces.
- **Collagen vascular disease pneumonitis** has varied patterns of parenchymal and pleural involvement. The prognosis is poor.

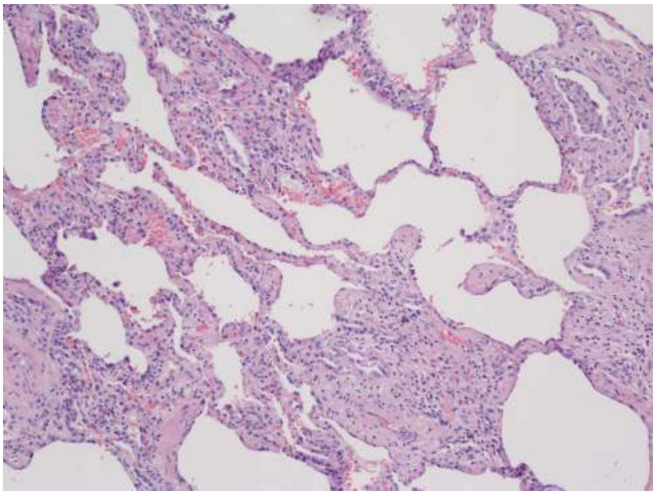

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Figure 14-5. Honeycomb Lung (Idiopathic Pulmonary Fibrosis)

- **Smoking-related pneumonitis.** Several entities have been identified.
  - Desquamative interstitial pneumonia features alveolar macrophages.
  - Respiratory bronchiolitis features bronchiolocentric macrophages.
  - Smoking-related interstitial fibrosis shows septal collagen deposition without significant associated inflammation.
- **Hypersensitivity pneumonitis.** After exposure to a sensitizing agent such as moldy hay, patients present with a febrile acute reaction or a chronic disease with weight loss. Biopsy shows peribronchiolar acute and chronic interstitial inflammation +/- noncaseating granulomas. The disease is immunologically mediated.
- **Eosinophilic pneumonia** describes a group of diseases with varying clinical features but a common histologic finding of a mixed septal inflammatory infiltrate and eosinophils within alveolar spaces. **Loeffler's syndrome** is a self-limiting type of eosinophilic pneumonia with peripheral blood eosinophilia.

Occupation-associated pneumoconiosis is a common cause of chronic interstitial lung disease. It is considered separately here to show the full spectrum of disease since neoplasia may occur during its course.

- **Pneumoconioses** are fibrosing pulmonary diseases caused by inhalation of an aerosol (mineral dusts, particles, vapors, or fumes). Key factors affecting their development include the type of aerosol and its ability to stimulate fibrosis; the dose and duration of exposure; and the size of the particle, with only particles <10 microns entering the alveolar sac.
  - **Coal worker's pneumoconiosis** is an important pneumoconiosis that is due to anthracosis, in which carbon pigment (anthracotic pigment) from coal mining accumulates in macrophages along the pleural lymphatics and interstitium. Clinically, the disease may progress through several stages. The earliest stage is asymptomatic.
  - **Simple coal worker's pneumoconiosis** (black lung disease) is characterized by coal-dust macules and nodules in the upper lobes that produce little pulmonary dysfunction.

- **Complicated coal worker's pneumoconiosis** is characterized by progressive massive fibrosis that is accompanied by increasing respiratory distress, secondary pulmonary hypertension, and cor pulmonale.
- **Caplan syndrome** is the term when pneumoconiosis (of any type) accompanies rheumatoid arthritis.

- **Asbestosis** is caused by members of a family of crystalline silicates. Occupations in which asbestos exposure may occur include shipyard work, insulation and construction industries, brake-lining manufacture.
  - **Serpentine asbestos** is composed of curved, flexible fibers, with the most common type of serpentine asbestos being chrysotile.
  - **Amphibole asbestos** is composed of straight, brittle fibers. Important types include crocidolite, tremolite, and amosite. Amphibole asbestos is more pathogenic than serpentine asbestos, and is highly associated with mesotheliomas.
  - **The pulmonary pathology of asbestosis** is a diffuse interstitial fibrosis that begins in the lower lobes; it causes slowly progressive dyspnea which may eventually be complicated by secondary pulmonary hypertension and cor pulmonale. Pulmonary biopsy may demonstrate asbestos bodies that have become coated with iron (ferruginous bodies). Otherwise, the findings are the same as usual interstitial pneumonia.
  - **Pleural involvement** may take the form of parietal pleural plaques (acellular type I collagen deposition) in a symmetrical distribution involving the domes of the diaphragm and posterolateral chest walls on chest x-ray. The apices and costophrenic angles are spared. Plaques on the anterior chest wall may be seen on CT. Fibrous pleural adhesions may occur on the visceral pleura.
  - **Lung cancer** is the most common tumor in asbestos-exposed individuals; there is a strong synergistic effect between smoking and asbestos exposure.
  - **Malignant mesothelioma** is a rare, highly malignant neoplasm associated with occupational exposure to asbestos in 90% of cases. It presents with recurrent pleural effusions, dyspnea, and chest pain. The tumor grossly encases and compresses the lung; microscopic exam exhibits carcinomatous and sarcomatous elements (biphasic pattern), while electron microscopy shows long, thin microvilli on some tumor cells. The prognosis of mesothelioma is poor. Other problems include increased risk of laryngeal, stomach, and colon cancers. Family members also have increased risk of cancer due to the worker bringing home clothing covered with asbestos fibers.

- **Silicosis** is due to exposure to silicon dioxide (silica). It is seen most frequently with occupational exposure (sandblasters, metal grinders, miners). The **pulmonary pathology** shows dense nodular fibrosis of the upper lobes which may progress to massive fibrosis; birefringent silica particles can be seen with polarized light.

  Patients present with insidious onset of dyspnea that is slowly progressive despite cessation of exposure. X-ray shows fibrotic nodules in the upper zones of the lungs. There is an increased risk of TB.

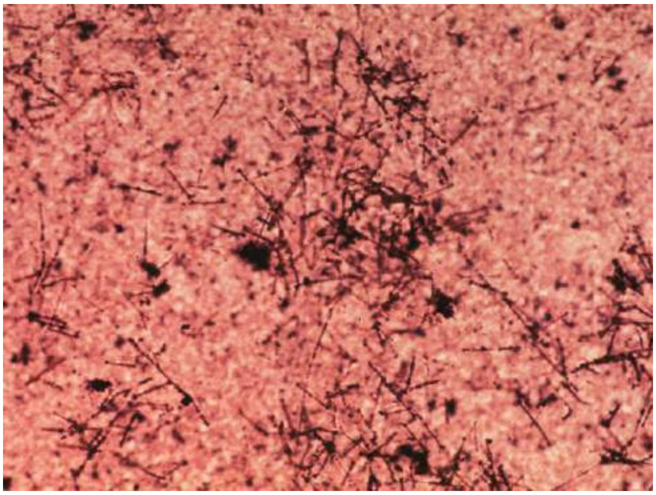

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Figure 14-6. Asbestos Fibers in Lung Tissue

- **Berylliosis** is an allergic granulomatous reaction due to workplace exposure to beryllium in the nuclear, electronics, and aerospace industries. Genetic susceptibility appears to play a role, as does a type IV hypersensitivity reaction, resulting in granuloma formation. Clinically, acute exposure causes acute pneumonitis, while chronic exposure causes pulmonary noncaseating granulomas and fibrosis, hilar lymph node granulomas, and systemic granulomas

VASCULAR DISORDERS

Pulmonary edema is fluid accumulation within the lungs, usually due to imbalance of Starling forces or endothelial injury.

- Pulmonary edema due to **increased hydrostatic pressure** can be seen in left-sided heart failure, mitral valve stenosis, high altitude pulmonary edema, and fluid overload.
- Pulmonary edema due to **decreased oncotic pressure** can be seen in nephrotic syndrome and liver disease.
- Pulmonary edema due to **increased capillary permeability** can be due to infections, drugs (bleomycin, heroin), shock, and radiation.

The pathology grossly shows wet, heavy lungs (usually worse in lower lobes), while microscopic examination shows intra-alveolar fluid, engorged capillaries, and hemosiderin-laden macrophages (heart-failure cells).

Pulmonary emboli (PE) and pulmonary infarction (*See* Circulatory Pathology, chapter 5.)

Pulmonary hypertension is increased pulmonary artery pressure, usually due to increased vascular resistance or blood flow.

The etiology varies and can include chronic obstructive pulmonary disease and interstitial disease (hypoxic vasoconstriction); multiple ongoing pulmonary emboli; mitral stenosis and left heart failure; congenital heart disease with left to right shunts (atrial septal defect, ventricular septal defect, patent ductus arteriosus); and primary (idiopathic) pulmonary hypertension, typically in young women.

Note

High altitude pulmonary edema is a hydrostatic-type pulmonary edema. Symptoms (i.e., cough and shortness of breath) resolve with descent and administration of oxygen.

Clinical Correlate

Dietary drugs fenfluramine and phentermine have been associated with primary pulmonary hypertension.

The pathology includes pulmonary artery atherosclerosis, small artery medial hypertrophy and intimal fibrosis, and plexogenic pulmonary arteriopathy. Pulmonary hypertension may also damage the heart, leading to right ventricular hypertrophy and then failure (cor pulmonale).

PULMONARY NEOPLASIA

Lung cancer is the leading cause of cancer death among both men and women; it has been increasing in women (increased smoking) in the past few decades. It occurs most commonly age 50–80. Major risk factors include cigarette smoking, occupational exposure (asbestosis, uranium mining, radiation, etc.), passive smoking, and air pollution. Clinical features include cough, sputum production, weight loss, anorexia, fatigue, dyspnea, hemoptysis, and chest pain. Obstruction may produce focal emphysema, atelectasis, bronchiectasis, or pneumonia.

Common genetic mutations in lung cancer involve the oncogenes *MYCL* (small cell carcinomas) and *KRAS* (adenocarcinomas); tumor suppressor genes: *TP53* and *RB1*.

Adenocarcinoma is more commonly seen in women and nonsmokers. Grossly, it causes a peripheral gray-white mass, and the tumor may develop in areas of parenchymal scarring (scar carcinoma). Microscopically, common patterns include acinar, papillary, mucinous, and solid. The precursor lesion—atypical adenomatous hyperplasia—progresses to adenocarcinoma in situ (noninvasive well-differentiated tumor <3 cm) and to minimally invasive tumor (invasion no more than 5 mm) before progressing to invasive adenocarcinoma.

Squamous cell carcinoma (SCC) is strongly related to smoking and affects males more than females. Squamous cell carcinoma arises from bronchial epithelium after a progression:

metaplasia → dysplasia → carcinoma *in situ* → invasive carcinoma

Pathologically, the tumor grossly causes a gray-white bronchial mass, usually centrally located. Microscopically, well-differentiated tumors show invasive nests of squamous cells with intercellular bridges (desmosomes) and keratin production (“squamous pearls”).

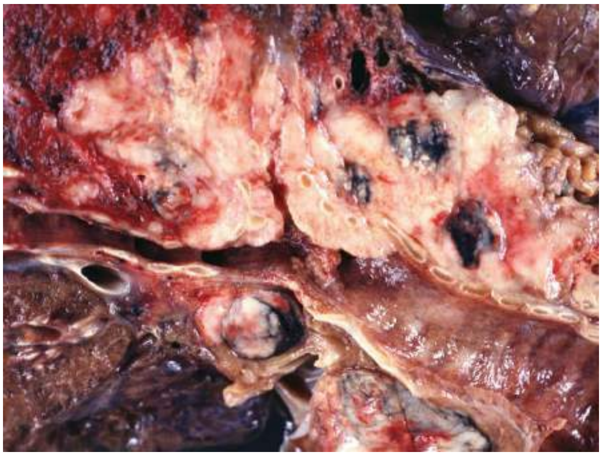

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Figure 14-7. Squamous Cell Carcinoma of the Lung

Small cell carcinoma has a strong association with smoking, and affects males more than females. This neuroendocrine tumor is very aggressive, with rapid growth and early dissemination. Small cell carcinoma is commonly associated with paraneoplastic syndromes.

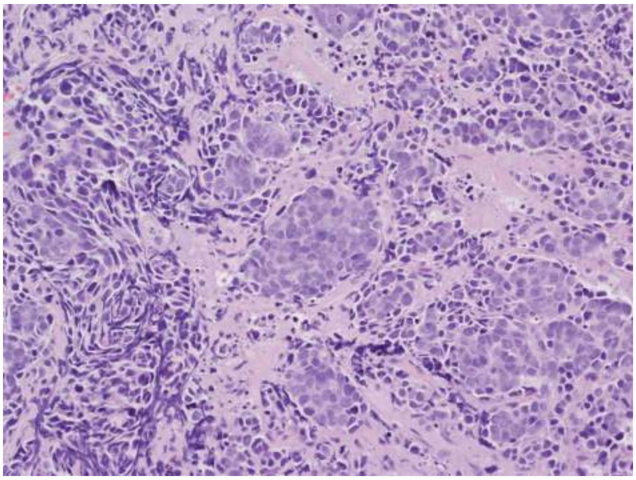

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Figure 14-8. Small Cell Carcinoma with Crush Artifact in the Lower Left Field

Pathologically, gross examination demonstrates central, gray-white masses. Microscopic examination shows small round or polygonal cells in clusters, and electron microscopy shows cytoplasmic dense-core neurosecretory granules.

Large cell carcinoma has large anaplastic cells without evidence of differentiation.

Intrathoracic spread of lung cancer is to lymph nodes, particularly hilar, bronchial, **tracheal, and mediastinal; pleura (adenocarcinoma);** and lung apex causing **Horner syndrome (Pancoast tumor)**.

- Obstruction of the superior vena cava by tumor causes **superior vena cava syndrome**, characterized by distended head and neck veins, plethora, and facial and upper arm edema.
- **Esophageal obstruction** can cause dysphagia.
- Recurrent laryngeal **nerve involvement** causes hoarseness, while phrenic nerve damage causes diaphragmatic paralysis.

Extrathoracic sites of metastasis include adrenal (>50%), liver, brain, and bone.

Note

Horner Syndrome causes ipsilateral:

- Ptosis
- Miosis
- Anhidrosis
- Enophthalmos

Paraneoplastic syndromes

- Endocrine/metabolic syndromes include Cushing syndrome secondary to ACTH production, SIADH secondary to ADH production, and hypercalcemia secondary to PTH production (squamous cell carcinoma).
- Eaton-Lambert syndrome (*See* Skeletal Muscle and Peripheral Nerve Pathology chapter.)
- Acanthosis nigricans (*See* Skin Pathology chapter.)
- Hypertrophic pulmonary osteoarthropathy is characterized by periosteal new bone formation with clubbing and arthritis.

Treatment of non–small cell lung cancer is with surgery, and treatment of small cell lung cancer is with chemotherapy and radiation. Despite treatment, the prognosis is poor, with overall 5-year survival 16%.

Bronchial carcinoids occur in a younger age group (mean age 40 years) and typically produce a polypoid intrabronchial mass or plaque; it is characterized on light microscopy by small, round, uniform cells growing in nests (organoid pattern), and on electron microscopy by cytoplasmic dense-core neurosecretory granules. Atypical carcinoid is more aggressive than typical carcinoid.

Metastatic carcinoma is the most common malignant neoplasm in the lung. It typically causes multiple, bilateral, scattered nodules; common primary sites include breast, stomach, pancreas, and colon.

Hamartomas are benign tumors; they occur more commonly in middle-aged adults but also occur in children. They can appear as coin lesions on chest x-ray. Microscopically, they are comprised of nonencapsulated fibromyxoid tissue. Carney triad is the finding of a hamartoma with a predominantly cartilaginous component (pulmonary chondroma), an extra-adrenal paraganglioma and a gastric gastrointestinal stromal tumor.

Bridge to Pharmacology

Erlotinib (Tarceva®) is used to treat non–small cell lung cancer, pancreatic cancer, and other types of cancers that have failed a prior trial of chemotherapy. It is a tyrosine-kinase inhibitor which inhibits the epidermal growth factor receptor (EGFR). The drug targets EGFR tyrosine kinase which is highly expressed and sometimes mutated in various forms of cancer.

LARYNGEAL CANCER

Laryngeal squamous cell carcinoma causes hoarseness, difficulty swallowing, pain, hemoptysis, and eventual respiratory compromise. Risk factors include smoking, alcohol, and frequent cord irritation (professional singing or lecturing). Complications include direct extension, metastases, and infection.

DISEASES OF THE PLEURAL CAVITY

Pleural effusion is the accumulation of fluid in the pleural cavity.

- *Empyema* refers to pus in pleural space.
- *Chylothorax* refers to chylous fluid in the pleural space secondary to obstruction of the thoracic duct, usually by tumor.

Pneumothorax is the term used for air in the pleural cavity. It can be due to traumatic penetrating chest wall injuries or spontaneous rupture of apical blebs in typically tall young adults (spontaneous pneumothorax). The term **tension pneumothorax** is used if a life-threatening shift of thoracic organs across midline occurs.

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Figure 14-9. Densely Black Appearance on Chest X-Ray of a Pneumothorax

Hemothorax is the presence of blood in the pleural cavity. Trauma is a common cause. There may be hypotension and shift of the trachea to the unaffected side.

Chylothorax is lymphatic fluid in the pleural cavity. Malignancy is a common cause.

Mesothelioma (*See* section on asbestosis earlier in this chapter.)

Renal Pathology 15

Learning Objectives

- ❑ Demonstrate understanding of congenital anomalies of the kidney
- ❑ Use knowledge of cystic disease to solve problems
- ❑ Answer questions about nephritic/nephrotic syndrome, secondary/chronic glomerulonephritis, tubulointerstitial nephritis, and acute tubular injury
- ❑ Describe epidemiology and course of urolithiasis
- ❑ Solve problems concerning chronic renal failure
- ❑ Solve problems concerning tumors of the kidney
- ❑ Explain information related to ureteral disorders
- ❑ Explain information related to urinary bladder pathology

CONGENITAL ANOMALIES OF THE KIDNEY

Renal agenesis

- **Bilateral agenesis** is incompatible with life. Ultrasound shows oligohydramnios.
- Affected fetuses typically also have Potter facies (flattened nose, posteriorly rotated ears, and recessed chin); talipes equinovarus (talus [ankle]+ pes [foot] and equino [heel] + varus [turned upward] = clubfoot); and pulmonary hypoplasia.
- In **unilateral agenesis**, the remaining kidney undergoes compensatory hypertrophy. Patients often have adequate renal function and are asymptomatic.

Hypoplasia is failure of a kidney (usually unilateral) to develop to normal weight; the hypoplastic kidney has a decreased number of calyces and lobes.

Horseshoe kidney is a common congenital anomaly that is found in 1 in 600 abdominal x-rays. The kidneys show fusion, usually at the lower pole; affected individuals have normal renal function but may be predisposed to renal calculi.

Abnormal locations. The most common abnormal location is a pelvic kidney. The ectopic kidney usually has normal function. Tortuosity of ureters may predispose to pyelonephritis.

Note

Oligohydramnios (Potter) Sequence

Renal agenesis
↓
Oligohydramnios
↓
Fetal compression
↓
Flattened facies and positional abnormalities of hands and feet

CYSTIC DISEASE

Autosomal recessive polycystic kidney disease (also called infantile polycystic kidney disease or renal dysgenesis Potter type I) is a rare autosomal recessive disease that presents in infancy with progressive and often fatal renal failure. A mutation in the *PKHD1* gene is implicated.

The kidneys are bilaterally enlarged and have a spongelike cut surface. The liver may have multiple hepatic cysts and cirrhosis may develop in childhood. Pulmonary hypoplasia is present to varying degrees.

Autosomal dominant polycystic kidney disease (also called adult polycystic kidney disease or renal dysgenesis Potter type III) is an autosomal dominant disease that affects 1 in 1,000. There is most frequently a mutation of the *PKD1* gene on chromosome 16 which produces a transmembrane protein called polycystin 1. Other mutations involve *PKD2* and polycystin 2.

Clinically, patients are asymptomatic with normal renal function until middle age, and then present with renal insufficiency, renal stones, hematuria, and hypertension or with abdominal masses and flank pain. Diagnosis is established with U/S and CT scan. Most patients develop end-stage renal failure by decade 7.

On gross pathologic examination, the kidneys have massive bilateral enlargement with large bulging cysts filled with serous, turbid, or hemorrhagic fluid. Microscopic examination shows functioning nephrons present between the cysts; the cysts arise from the tubular epithelial cells of the kidney.

Extrarenal manifestations include cysts of the liver, pancreas, and lungs; berry aneurysms of the circle of Willis; mitral valve prolapse; and colonic diverticula.

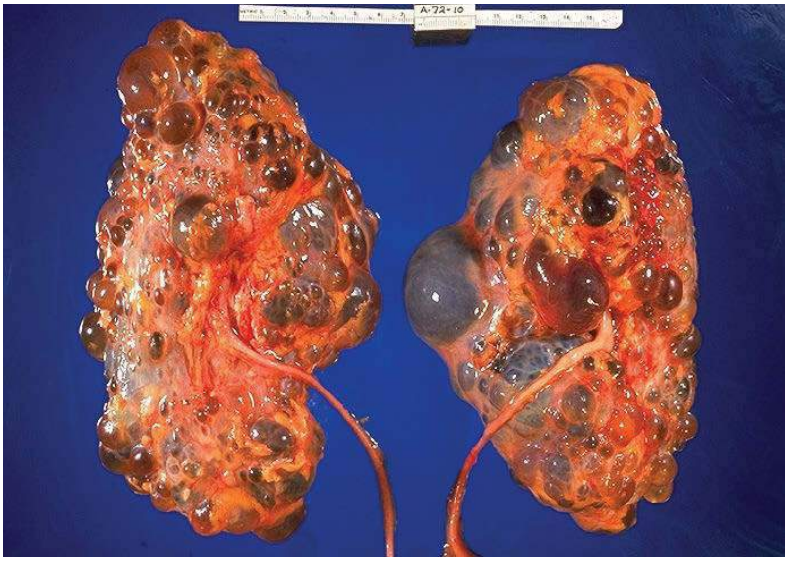

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Figure 15-1. Gross Pathology of Polycystic Kidneys

Renal dysplasia is the most common renal cystic disease in children, in whom it causes an enlarged renal mass with cartilage and immature collecting ducts. It may progress clinically to renal failure.

Acquired polycystic disease is seen in renal dialysis patients and is associated with a small risk of developing renal adenomas and renal cell carcinoma.

Simple retention cysts of the kidney are common in adults and occasionally cause hematuria.

Medullary diseases with cysts

- Medullary sponge kidney can cause nephrolithiasis but is otherwise innocuous.
- Nephronophthisis-medullary cystic disease complex presents as polyuria and polydipsia; it can progress to chronic renal failure in young adults. There are autosomal recessive forms. The cysts are in the cortex and the medulla.

GLOMERULAR DISEASES

Immune mechanisms play a role in the pathogenesis of most glomerular diseases, either via deposition of immune complexes or injury from antibodies. Glomerular diseases may be divided into those originating in the kidney and those caused by systemic disease (secondary).

Glomerular disease may present clinically as **nephrotic** or **nephritic syndrome**. Their clinical features are different.

Table 15-1. Clinical Syndromes in Glomerular Disease

Nephritic Syndrome	Nephrotic Syndrome
Hematuria (RBC casts)	Severe proteinuria (>3.5 g/day)
Hypertension	Hypoalbuminemia (<3 g/dL)
Azotemia	Generalized edema
Oliguria	Hyperlipidemia
Proteinuria (<3.5 g/day)	Lipiduria

Renal biopsy can yield a definitive diagnosis when light microscopy features are considered in concert with immunofluorescence (IF) and electron microscopy (EM).

Clinical Correlate

The cysts in autosomal dominant (adult) PKD involve <10% of nephrons, but they gradually expand and compress the rest of the kidney, interfering with its function. This is why kidney function can remain normal for many years.

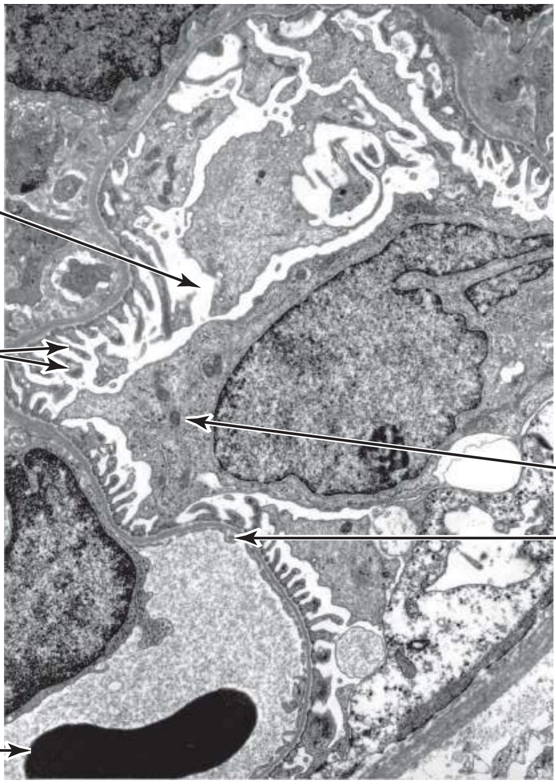

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Figure 15-2. Transmission Electron Micrograph Demonstrating Podocytes

PRIMARY GLOMERULOPATHIES (NEPHRITIC SYNDROME)

Acute poststreptococcal glomerulonephritis (APSGN) (or acute proliferative glomerulonephritis or postinfectious glomerulonephritis) is an immune complex disease that typically occurs 2–4 weeks after a streptococcal infection of the throat or skin. There is a decreasing incidence in the United States; children are affected more often than adults.

Clinical Correlate

The classic presentation of APSGN is a young child with fever, malaise, periorbital edema, hypertension, smoky urine, and oliguria, beginning approximately 2 weeks after a streptococcal infection.

The infecting organism is most commonly β-hemolytic group A *streptococci*, but APSGN can also be caused by other bacteria, viruses, parasites, and even systemic diseases (SLE and polyarteritis nodosa). Clinically, it presents with nephritic syndrome with elevated antistreptolysin O (ASO) titers (when related to streptococcal infection) and low C3.

Renal biopsy. Light microscopy shows an infiltrate of neutrophils in the glomeruli; the process is diffuse, that is, it involves all the glomeruli. Immunofluorescence shows granular deposits of IgG and C3 throughout the glomerulus within the capillary walls and some mesangial areas. Electron microscopy shows subepithelial immune complex deposits (humps).

Note

The characteristic finding in RPGN is the formation of crescents within Bowman space. The crescents are composed of fibrin, parietal epithelial cells, monocytes, and macrophages.

Treatment is conservative fluid management. Most children (>95%) have a good prognosis, with complete recovery, but severe disease can also occur (RPGN 1% and chronic glomerulonephritis 2%). In adults, 15-50% develop end-stage disease.

Rapidly progressive glomerulonephritis (RPGN) (crescentic glomerulonephritis) is group of diseases characterized by glomerular crescents and a rapid deterioration of renal function.

- **Anti-glomerular basement membrane antibody-mediated crescentic glomerulonephritis** has a peak incidence at age 20-40, and males are affected more frequently than females.

Pulmonary involvement is called **Goodpasture syndrome**. These patients have pulmonary hemorrhage and hemoptysis.

Renal biopsy findings include hypercellularity, crescents, and fibrin deposition in glomeruli. Immunofluorescence shows a smooth and linear pattern of IgG and C3 in the glomerular basement membrane (GBM). By electron microscopy, there are no deposits, but there is glomerular basement membrane disruption.

Even with treatment (plasma exchange, steroids, and cytotoxic drugs), the prognosis is poor because of risks of severe and life-threatening pulmonary hemorrhage and renal failure. Early aggressive treatment may prevent end-stage renal failure.

Immune-complex mediated crescentic glomerulonephritis

- Any of the immune complex nephritides can cause crescent formation.
- IF shows a granular pattern and EM shows discrete deposits.

Pauci-immune crescentic glomerulonephritis

- Antineutrophilic cytoplasmic antibodies (ANCA) are present in serum.
- IF and EM are negative for immunoglobulins and complement components.

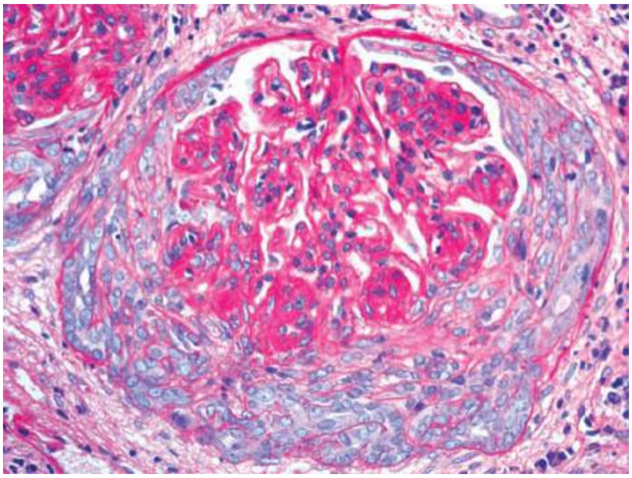

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Figure 15-3. Crescent Formation in Rapidly Progressive Glomerulonephritis, as Seen with Trichrome Stain

IgA nephropathy (Berger disease) is the most common cause of glomerulonephritis in the world, being particularly common in France, Japan, Italy, and Austria. It affects children and young adults (mostly males).

IgA nephropathy is characterized by recurrent gross hematuria (a predominately nephritic presentation), whose onset may follow a respiratory infection. IgA nephropathy can be associated with celiac sprue and Henoch-Schönlein purpura or can be secondary to celiac sprue or liver disease.

The pathogenesis is unknown, but may be related to a possible entrapment of circulating immune complexes with activation of the alternate complement pathway; it may also be related to a genetic predisposition.

Renal biopsy. Light microscopy may show normal glomeruli or mesangial proliferation. Immunofluorescence shows mesangial deposits of IgA and C3. Electron microscopy shows mesangial immune complex deposits.

Many cases slowly progress to renal failure over 25 years.

Membranoproliferative glomerulonephritis (MPGN) is a form of glomerular disease that affects both the glomerular mesangium and the basement membranes; it occurs in 2 types, Type I and Type II (dense deposit disease).

The clinical presentation is variable, and may be nephritic, nephrotic, or mixed. MPGN may be secondary to many systemic disorders (systemic lupus erythematosus, endocarditis), chronic infections (HBV, HCV, HIV), and malignancies (chronic lymphocytic leukemia).

Laboratory studies show decreased serum C3 and the presence of C3 nephritic factor (MPGN type II).

Light microscopy demonstrates a lobulated appearance of the glomeruli due to mesangial and endothelial cell proliferation and/or deposition of subendothelial immune complex deposits. Splitting of the basement membrane ("tram-tracking") may be seen with a silver or periodic acid-Schiff (PAS) stain.

Immunofluorescence in type I MPGN shows a granular pattern of C3 often with IgG, C1q, and C4; in type II, immunofluorescence shows a granular and linear pattern of C3 deposition.

Electron microscopy in type I shows subendothelial and mesangial immune complex deposits, and in type II shows dense deposits within the glomerular basement membrane.

MPGN typically has a slowly progressive course, resulting in chronic renal failure over the course of 10 years. There is a high incidence of recurrence in transplants.

Alport syndrome is a rare X-linked disorder caused by a defect in type IV collagen. It is characterized by hereditary nephritis, hearing loss, and ocular abnormalities. The most common mutation causing Alport is in the *COL4A5* gene coding for the alpha-3, -4, and -5 chain of type IV collagen.

Gross or microscopic hematuria begins in childhood. Hearing loss (leading to sensorineural deafness) and various ocular abnormalities of the lens and cornea can occur. Alport is a progressive disease that ultimately results in renal failure.

Electron microscopy shows alternating thickening and thinning of basement membrane with splitting of the lamina densa, causing a "basketweave" appearance.

Note

Henoch-Schönlein Purpura

- Systemic childhood disorder
- Onset often follows URI
- IgA nephropathy
- Abdominal pain
- GI bleeding
- Arthralgia
- Palpable purpura on legs and buttocks

Note

Most patients with MPGN type II disease have an autoantibody called C3 nephritic factor. This antibody stabilizes C3 convertase, which leads to enhanced degradation and low serum levels of C3.

Note

"Tram-Tracking"

This double contour appearance is caused by the splitting of the GBM by extension of the mesangial cell processes into the capillary loop.

PRIMARY GLOMERULOPATHIES (NEPHROTIC)

Membranous glomerulonephritis is a common cause of nephrotic syndrome in adults that is mediated by immune complexes.

Most cases (85%) are idiopathic; in most of these cases, autoantibodies cross-react with podocyte antigens. Membranous glomerulonephritis may also be caused by drugs (penicillamine), infections (hepatitis virus B and C, syphilis, etc.), and systemic diseases (SLE, diabetes mellitus, etc.). It has also been associated with malignant carcinomas of the lung and colon, and there may be a genetic predisposition.

Renal biopsy shows diffuse thickening of the capillary walls. Basement membrane projections ("spikes") are seen on silver stains. Immunofluorescence shows a granular and linear pattern of IgG and C3. Electron microscopy shows subepithelial deposits along the basement membranes with effacement of podocyte foot processes.

The clinical course is variable and may lead to spontaneous remission, persistent proteinuria, or end-stage renal disease.

Minimal change disease (also called lipoid nephrosis and nil disease) is the most common cause of nephrotic syndrome in children. Peak incidence is age 2–6.

The diagnosis is one of exclusion. Light microscopy shows normal glomeruli with lipid accumulation in proximal tubule cells (lipoid nephrosis). Immunofluorescence is negative, with no immune deposits. Electron microscopy shows effacement of epithelial (podocyte) foot processes, microvillous transformation, and no immune complex deposits.

The prognosis is excellent because treatment with corticosteroids produces a dramatic response in children. The majority have a complete recovery.

Focal segmental glomerulosclerosis is a very common cause of nephrotic syndrome that occurs in all ages. African Americans are affected more frequently than Caucasians.

Note

Focal: only some of the glomeruli are affected

Segmental: only a portion of the glomerular tuft exhibits sclerosis

The condition may be idiopathic (primary), or it may be related to a wide variety of predisposing conditions including loss of renal tissue; preexisting glomerular diseases (such as IgA nephropathy); sickle cell anemia; heroin use; AIDS; or morbid obesity. Inherited and congenital forms occur.

Renal biopsy. Light microscopy shows focal segmental sclerosis and hyalinization of glomeruli; focal segmental glomerulosclerosis initially affects the glomeruli along the medullary border. Immunofluorescence shows IgM and C3 deposits in the sclerotic segments. Electron microscopy shows effacement of foot processes in nonsclerotic regions and increased mesangial matrix in sclerotic segments.

Clinical course. There is frequently a poor response to steroids, with the overall prognosis being poor (most progressing to chronic renal failure), though children do better than adults. There is a high rate of recurrence in renal transplants. A variant form, collapsing glomerulopathy, has a worse prognosis.

SECONDARY GLOMERULONEPHRITIS

Secondary glomerulonephritis is glomerular disease that is secondary to other disease processes.

Diabetes causes nodular glomerulosclerosis, hyaline arteriolosclerosis, and diabetic microangiopathy. Clinically, diabetic patients may develop microalbuminuria that can progress to nephrotic syndrome.

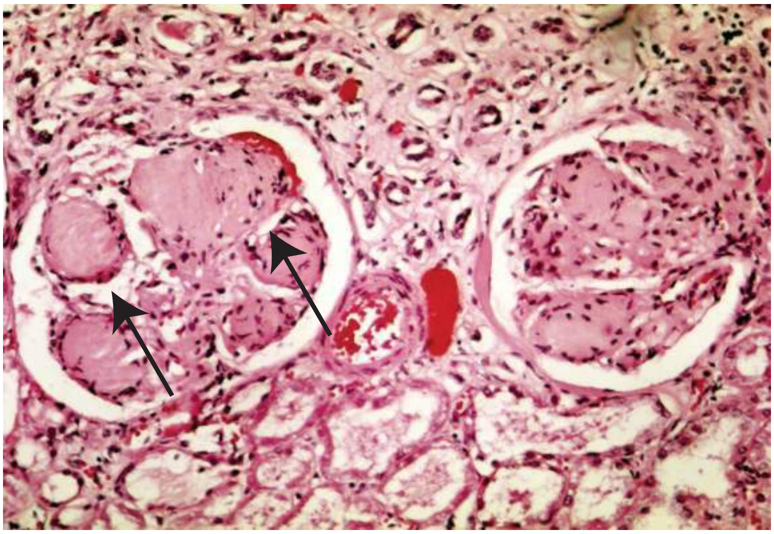

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Figure 15-4. Sclerotic Nodules (arrows) of Nodular Glomerulosclerosis (Kimmelstiel-Wilson Syndrome) Associated with Diabetes

Systemic lupus erythematosus can cause various patterns of damage to the kidney with clinical features that can include hematuria, nephritic syndrome, nephrotic syndrome, hypertension, and renal failure.

CHRONIC GLOMERULONEPHRITIS

End-stage renal disease is the final stage of many forms of glomerular disease. It is characterized by progressive renal failure, uremia, and ultimately death.

Clinical features include anemia, anorexia, malaise, proteinuria, hypertension, and azotemia. Urinalysis shows broad, waxy casts. On pathologic examination, the kidneys are grossly small and shrunken; microscopic exam shows hyalinization of glomeruli, interstitial fibrosis, atrophy of tubules, and a lymphocytic infiltrate.

Treatment is dialysis and renal transplantation.

TUBULOINTERSTITIAL NEPHRITIS

Tubulointerstitial nephritis is an acute or chronic inflammation of tubules and interstitium. It can be due to many causes, including medications, infections, acute pyelonephritis, systemic lupus erythematosus, lead poisoning, urate nephropathy, or multiple myeloma.

- **Acute pyelonephritis** refers to bacterial infections involving the renal pelvis, tubules, and interstitium. Pyelonephritis affects females much more than males, but the incidence increases in older males with prostatic hyperplasia.

  Ascending infection is the most common route of infection. Causative organisms include gram-negative enteric bacilli, *Escherichia coli, Proteus, Klebsiella,* and *Enterobacter*. Predisposing factors include urinary obstruction, vesicoureteral reflux, pregnancy, urethral instrumentation, diabetes mellitus, benign prostatic hyperplasia, and other renal pathology. Symptoms can include fever, chills, and malaise; dysuria, frequency, and urgency; and costovertebral angle tenderness. Urinalysis shows pyuria and white blood cell casts.

  The kidney may be enlarged, and the cortical surface may show abscesses. Microscopically there is a neutrophilic interstitial infiltrate. **Parenchymal abscesses** may be present. The tubules contain neutrophil casts.

- **Chronic pyelonephritis** can occur from chronic obstruction or in the setting of vesicoureteral reflux. Scarring can be seen at the upper and lower poles of the kidney, with associated calyceal blunting. Microscopically there is interstitial fibrosis and inflammation with thyroidization of the tubule. Some patients develop glomerulosclerosis. Renal insufficiency develops gradually.
- **Drug-induced tubular interstitial nephritis** is commonly caused by penicillins, other antibiotics, diuretics, and NSAIDs. Interstitial inflammation is characteristic and granulomas may be seen. This hypersensitivity reaction presents a couple of weeks after drug exposure with fever, eosinophilia, rash, and hematuria. Minimal proteinuria may be present. Recovery is expected after withdrawal of the drug.
- **Urate nephropathy** is caused by a deposition of urate crystals (secondary to leukemia treatment, lead poisoning, and gout) in renal tubules and interstitium. It may produce acute renal failure.

Clinical Correlate

It may be difficult to distinguish cystitis from pyelonephritis. The presence of fever, costovertebral angle tenderness, and WBC casts in the urine are helpful clues to the diagnosis of pyelonephritis.

ACUTE TUBULAR INJURY

Acute tubular injury (ATI) is acute renal failure associated with potentially reversible injury to the tubular epithelium. It is the most common cause of acute renal failure in the United States. It is characterized by oliguria with elevation of blood urea nitrogen (BUN) and creatinine; metabolic acidosis and hyperkalemia; and dirty brown granular casts and epithelial casts on urinalysis.

- **Ischemic acute tubular necrosis** is the most common cause of ATI. The condition is due to decreased blood flow caused by severe hemorrhage, severe renal vasoconstriction, hypotension, dehydration, or shock.
- **Nephrotoxic ATN** has a large number of causes, including drugs (e.g., polymyxin, methicillin, gentamicin, sulfonamides); radiographic contrast agents; heavy metals (e.g., mercury, lead, gold); organic solvents (e.g., carbon tetrachloride, chloroform, methyl alcohol); ethylene glycol (antifreeze); mushroom poisoning; phenol; pesticides; and myoglobin.

The **prognosis** is excellent if the patient survives the underlying disease, and if the patient had no preexisting kidney disease.

UROLITHIASIS

Renal calculi occur in up to 6% of the population; men are affected more often than women.

Note

- **Calcium oxalate stones** form in people with alkaline urine.
- **Uric acid** and **cystine stones** form in people with acidic urine.

- **Stone composition.** Most (75%) stones are calcium oxalate stones. Magnesium ammonium phosphate ("struvite") stones are associated with infection by urea-splitting bacteria (*Proteus*), and these stones often form large staghorn calculi. Uric acid stones are seen in gout, leukemia, and in patients with acidic urine. Cystine stones are uncommon.

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Figure 15-5. Struvite (Magnesium Ammonium Phosphate) Stone Forming Staghorn Calculi

- **Pathology.** Most stones are unilateral stones that are formed in the calyx, pelvis, and urinary bladder.
- **Clinical features.** Calcium stones are radiopaque and can be seen on x-ray. Renal colic may occur if small stones pass into the ureters. Stones may cause hematuria, urinary obstruction, and predispose to infection.
- **Treatment of stones** is with lithotripsy or endoscopic removal.

CHRONIC RENAL FAILURE

Chronic renal failure is the end stage of many different renal diseases. It is characterized pathologically by bilaterally shrunken kidneys. Clinically, it causes progressive irreversible azotemia, normocytic anemia, platelet dysfunction, renal osteodystrophy, and hypertension.

VASCULAR DISORDERS OF THE KIDNEY

Renal artery stenosis of any etiology causes decreased blood flow to the involved kidney, with resulting secondary hypertension that is often not responsive to antihypertensive medications. Treatment is usually surgical.

- Atheromatous plaque is the most common cause of renal artery stenosis.
- Dysplastic lesions ("fibromuscular dysplasia") are an important additional cause of renal artery stenosis.

  All 3 lesion types occur in middle-aged adults.
  - Medial fibroplasia with aneurysms (most common) causes alternating stenosis and aneurysms in "string of beads" pattern on arteriography
  - Perimedial fibroplasia involves the outer media
  - Medial hyperplasia
- Miscellaneous diseases that can affect the renal arteries (with or without stenosis) include congenital anomalies, Takayasu arteritis, and radiation injuries.

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Figure 15-6. Renal Artery Stenosis as Demonstrated by Angiogram

Benign nephrosclerosis is caused by hypertension. The kidneys have a finely granular external surface and on microscopy show hyaline arteriolosclerosis, tubular atrophy, interstitial fibrosis, and glomerulosclerosis. Lab findings include mild proteinuria, hematuria, and azotemia.

Malignant (accelerated) hypertension can damage the kidney, causing fibrinoid necrosis of arterioles, glomerulitis, and hyperplastic arteriolosclerosis. Clinically, it causes cerebral edema, papilledema, retinal hemorrhage, intracerebral hemorrhage, and oliguric acute renal failure. The cortical surface shows pinpoint petechial hemorrhages ("flea-bitten" look).

Renal infarction is due to thrombi from the left side of the heart, atheroembolic disease, and vasculitis. It presents with sudden onset of flank pain and hematuria. Small infarcts may be asymptomatic.

Sickle cell anemia can cause medullary infarctions due to blockage of blood flow in the medullary vessels, which can result in asymptomatic hematuria, loss of urine concentrating ability, renal papillary necrosis, and pyelonephritis.

Diffuse cortical necrosis can cause anuria; the condition can occur with obstetric emergencies and disseminated intravascular coagulation.

TUMORS OF THE KIDNEY

Benign tumors of the kidney are as follows:

- **Cortical adenomas** are small, encapsulated cortical nodules measuring <3 cm; they are a common finding at autopsy. They may be composed of tubular or papillary structures. The papillary adenomas share the same chromosomal gains as papillary renal cell carcinoma.
- **Angiomyolipomas** are hamartomas composed of fat, smooth muscle, and blood vessels, common in patients with tuberous sclerosis.
- **Oncocytomas** are large, benign tumors that are resected to rule out renal cell carcinoma when they are found incidentally on imaging studies. They are brown on cut surface and have abundant pink cytoplasm on microscopy.

Renal cell carcinoma (RCC) is most common age 50–70, with males affected more than females.

Risk factors include cigarette smoking, chronic analgesic use, asbestos exposure, chronic renal failure, acquired cystic disease, and von Hippel-Lindau disease (VHL tumor suppressor gene).

In 10% of cases, the "classic" triad occurs:

- Hematuria
- Palpable mass
- Flank pain

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Figure 15-7. Renal Cell Carcinoma

A variety of paraneoplastic syndromes from ectopic hormone production can occur:

- Polycythemia (erythropoietin production)
- Hypertension (renin production)
- Cushing syndrome (corticosteroid synthesis)
- Hypercalcemia (PTH-like hormone)
- Feminization or masculinization (gonadotropin release)

Renal cell carcinoma may also cause secondary amyloidosis, a leukemoid reaction, or eosinophilia.

There is a high incidence of metastasis on initial presentation. The clinical course is unpredictable.

Gross examination typically demonstrates a large, solitary yellow mass found most commonly in the upper pole. Areas of necrosis and hemorrhage are commonly present. The tumor often invades the renal vein and may extend into the inferior vena cava and heart.

Histologic types of RCC are as follows:

- **Clear cell RCC (most common)**
    - Often invades renal venous system
    - May have loss of genetic material in 3p
    - A small percent occur in association with von Hippel-Lindau disease
    - Microscopically, there is an alveolar growth pattern with microcysts
    - Tumor is resistant to chemotherapy and radiotherapy
- **Papillary RCC**
    - Tends to be bilateral and multifocal
    - Cut surface is granular
    - Microscopically, the papillae have a single layer of cells
    - Gains of chromosome 7 and 17 are common
    - Duplications of chromosome 7 increase dosage of protooncogene *MET*
- **Chromophobe RCC (rare)**
    - Have cells that stain more darkly than clear cell RCC
    - Have loss of multiple chromosomes
    - Least aggressive of the RCCs

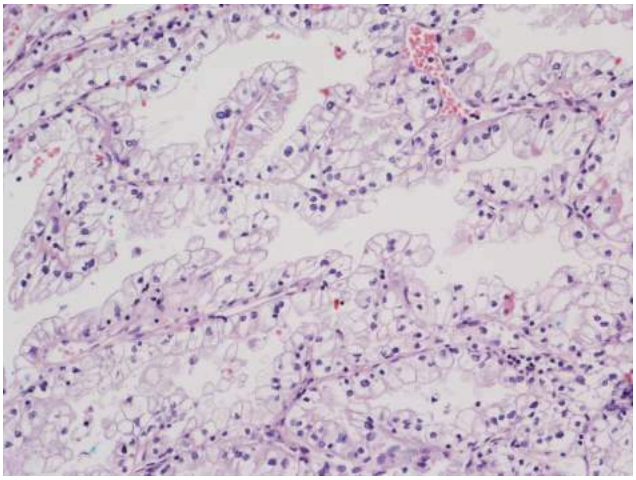

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Figure 15-8. Renal Cell Carcinoma

Wilms tumor (nephroblastoma) typically presents age 2-5 as a large abdominal mass. Patients with WAGR, DDS, or BWS syndrome are at increased risk of Wilms tumor.

- WAGR syndrome is the cluster of Wilms tumor, aniridia, genital anomalies, and mental retardation.
- Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder with characteristic features and an attendant increased risk of cancer.
- Denys-Drash syndrome (DDS) affects the genitalia and kidneys.

Both WAGR and DDS are associated with deletions and mutations, respectively, of the *WT1* gene. BWS arises through imprinting abnormalities at the *WT2* locus.

Pathologically, Wilms tumor causes a large, solitary tan mass. Microscopic examination reveals a tumor containing 3 elements: metanephric blastema, epithelial elements (immature glomeruli and tubules), and stroma.

Treatment is surgery, chemotherapy, and radiation, which as a combined therapy yields an excellent prognosis. Long-term survival rate is 90%.

Transitional cell carcinomas can involve the renal pelvis as well as the urinary bladder.

OBSTRUCTIVE DISORDERS OF THE URINARY SYSTEM

Hydronephrosis is a common complication of urinary tract obstruction. It is characterized by dilation of the ureter and renal pelvis. Specific causes include renal stones, retroperitoneal fibrosis, benign prostatic hyperplasia, and cervical cancer.

- In **unilateral hydronephrosis**, the kidney may enlarge to 20 cm. It may be found incidentally on physical exam. The parenchyma becomes compressed. If complete obstruction occurs suddenly, **necrosis of the renal papillae** may result.
- **Bilateral obstruction** that is complete presents as anuria; incomplete bilateral obstruction may present as polyuria.

URETERAL DISORDERS

Congenital anomalies include double ureters and congenital megaureter.

Ureteropelvic junction (UPJ) obstruction is the most common cause of hydronephrosis in infants and children. **Congenital UPJ obstruction** can also be identified in adults. It is often seen with other congenital anomalies. Treatment is surgical.

Ureteritis cystica describes chronic inflammation which causes formation of small mucosal cysts in the ureter. This condition can predispose for adenocarcinoma of the ureter.

Renal stones commonly lodge in the ureters.

Retroperitoneal fibrosis is usually an idiopathic condition causing severe fibrosis of the retroperitoneal area, which can entrap the ureters. Some cases show sclerosing conditions in other body sites and are associated with elevated serum IgG4.

Transitional cell carcinoma is the most common ureteral carcinoma.

URINARY BLADDER PATHOLOGY

Congenital anomalies of the bladder. Exstrophy of the bladder is a developmental failure of the formation of the abdominal wall and bladder which leaves the bladder open at the body surface. Urachal cyst remnants may permit drainage of urine from a newborn's umbilicus, and may also be a cause of bladder adenocarcinoma.

Cystitis. The etiology of cystitis varies, with important causes including organisms, notably from fecal flora (*Escherichia coli, Proteus, Klebsiella, Enterobacter*); radiation cystitis (may follow radiation therapy); and chemotherapy agents such as cyclophosphamide (hemorrhagic cystitis).

Clinically, it affects females far more than males. Symptoms include frequency, urgency, dysuria, and suprapubic pain; systemic signs such as fever and malaise are uncommon. Predisposing factors include benign prostatic hypertrophy, bladder calculi, and cystocele.

Malakoplakia is a bladder inflammatory pattern associated with a defect in macrophage function. The cause is unknown. Macrophages contain **Michaelis-Gutmann bodies**, laminated basophilic structures.

Urinary bladder tumors are most commonly due to transitional cell carcinoma. There is an increasing incidence of urinary bladder tumors; males are affected more than females, and peak incidence is age 40-60. Risk factors include:

- Cigarette smoking and occupational exposure to azo dye production (transitional cell carcinoma) (both due to 2-naphthylamine)
- Chronic bladder infection with *Schistosoma haematobium* (squamous cell carcinoma) (Africa including Egypt and the Middle East)

Bladder cancer usually presents with painless hematuria, but it may also cause dysuria, urgency, frequency, hydronephrosis, and pyelonephritis.

Prognosis of bladder cancer depends on the tumor grade and stage. There is a high incidence of recurrence.

Precursors of invasive transitional cell carcinoma can arise from a flat or papillary lesion.

- **Carcinoma in situ (CIS)** is a high-grade lesion with cytologic atypia in the full thickness of the epithelium. It is frequently multifocal. In 50-75% of untreated cases, it progresses to invasive cancer.
- Papillary precursors to invasive carcinoma include **papilloma ⇒ papillary urothelial neoplasia of low malignant potential ⇒ low-grade urothelial carcinoma ⇒ high-grade urothelial carcinoma**.

Other bladder tumors include papillomas, adenocarcinoma, and embryonal rhabdomyosarcoma.

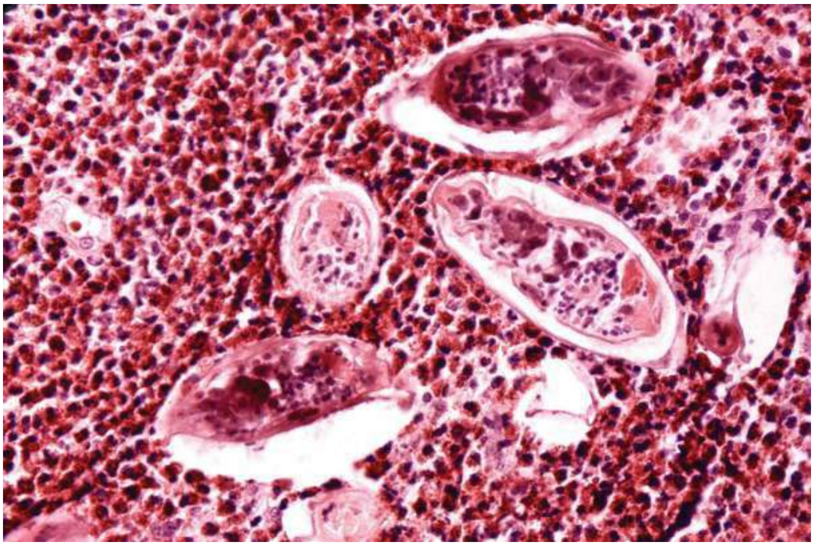

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Figure 15-9. Migratory Eggs of *Schistosoma haematobium* Surrounded by Dense Inflammation in the Bladder Wall

Miscellaneous bladder conditions

- **Acquired diverticula** can complicate urinary tract outlet obstruction due to benign prostatic hyperplasia or other causes.
- **Cystocele** is the term used for prolapse of the bladder into the vagina. It is common in middle-aged to elderly women.
- **Cystitis cystica et glandularis** causes formation of small cysts and glands in the bladder mucosa related to chronic inflammation. It is associated with an increased risk of adenocarcinoma.

Gastrointestinal Tract Pathology 16

Learning Objectives

- ❑ Answer questions about esophagus, stomach, small and large intestines
- ❑ Solve problems concerning gastric lymphoma

ESOPHAGUS

Congenital and Mechanical Disorders

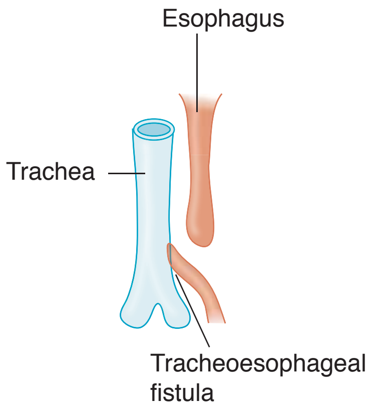
Tracheoesophageal fistula may arise as a congenital connection between the esophagus and trachea that is often associated with esophageal atresia. It is often discovered soon after birth because of aspiration. In adults the condition can occur secondary to malignancy, trauma, or iatrogenic causes.

Esophageal webs are web-like protrusions of the esophageal mucosa into the lumen which typically present with dysphagia. Plummer-Vinson syndrome is a disease of middle-aged women characterized by esophageal webs, iron deficiency anemia, and increased risk of carcinoma. Schatzki rings are web-like narrowings at the gastroesophageal junction.

Achalasia is a failure of the lower esophageal sphincter (LES) to relax with swallowing. The etiology is unknown in most cases; in South America, achalasia may be caused by Chagas disease. Presentation is with progressive dysphagia. The esophagus is characteristically dilated proximal to the lower esophageal sphincter; barium swallow shows a "bird-beak" sign. Microscopically, there is a loss of ganglion cells in the myenteric plexus. Treatment is LES balloon dilation or myotomy. Achalasia carries an increased risk for esophageal carcinoma.

Clinical Correlate

The most common type of tracheoesophageal fistula:



Clinical Correlate

Chagas disease, a tropical parasitic disease common in South America, is caused by *Trypanosoma cruzi*. It is transmitted by reduviid or "kissing" bugs. Chagas disease can cause:

- Romaña's sign (unilateral swelling of the eyelid)
- Cardiomyopathy
- Megaesophagus and megacolon

Hematemesis and Esophageal Bleeding

Mallory-Weiss syndrome is esophageal bleeding due to linear lacerations at the gastroesophageal junction from severe prolonged vomiting; the most common cause is acute alcohol ingestion and/or chronic alcoholism. Esophageal rupture (Boerhaave syndrome) may result.

Esophageal varices are dilated submucosal veins in the lower third of the esophagus, usually secondary to portal hypertension. The most common cause is cirrhosis. Clinically, the presentation is asymptomatic, though there is massive hematemesis when the varices are ruptured. Complications include potentially fatal hemorrhage. Treatment is generally band ligation, sclerotherapy, or balloon tamponade.

Esophagitis

Gastroesophageal reflux disease (reflux esophagitis) (GERD) is esophageal irritation and inflammation due to reflux of gastric secretions into the esophagus. Reflux typically presents with heartburn and regurgitation. Complications include bleeding, stricture, bronchospasm and asthma, and Barrett esophagus.

Barrett esophagus is a metaplasia of the squamous esophageal mucosa to a more protective columnar type (intestinal metaplasia). It occurs because of chronic exposure to gastric secretions, usually in the setting of GERD. The endoscopic appearance is of an irregular gastroesophageal junction with tongues of red granular mucosa extending up into the esophagus. Barrett esophagus has an increased risk for dysplasia and esophageal adenocarcinoma.

Note

Both **Mallory-Weiss tears** and **esophageal varices** are associated with alcohol abuse and can present with hematemesis. However,

- Mallory-Weiss tears typically occur acutely as a result of retching/vomiting.
- Esophageal varices result from portal hypertension and usually present with a more significant bleeding episode.

Note

The incidence of Barrett esophagus is increasing in the United States.

Esophageal Carcinoma

Squamous cell carcinoma (SCC) of the esophagus is the most common type of esophageal cancer in the world. Men > women, and African Americans > Caucasians; typical age is age >50. Risk factors include:

- Heavy smoking and alcohol use
- Achalasia
- Plummer-Vinson syndrome
- Tylosis
- Lye ingestion

Note

Tylosis is an autosomal dominant syndrome. The phenotypic hallmarks are oral leukoplakia and hyperkeratosis of the palms and soles. SCC of the esophagus is seen in up to 95% of affected individuals.

The presentation of squamous cell carcinoma of the esophagus varies; it is often asymptomatic until late in the course. When symptoms do develop they may include progressive dysphagia, weight loss and anorexia, bleeding, hoarseness, and cough. Diagnosis is by endoscopy with biopsy. Treatment is surgery though the prognosis is poor.

Adenocarcinoma of the esophagus affects Caucasians more than African Americans. It arises in the distal esophagus. The progression from Barrett metaplasia to dysplasia and eventually to invasive carcinoma occurs due to the stepwise accumulation of genetic and epigenetic changes. The prognosis is poor.

In the United States, adenocarcinoma and squamous cell carcinoma of the esophagus occur with equal frequency.

STOMACH

Congenital Disorders

Pyloric stenosis is a congenital stenosis of the pylorus due to marked muscular hypertrophy of the pyloric sphincter, resulting in gastric outlet obstruction. It affects male infants more than females. It is associated with Turner and Edwards syndromes. Presentation is the onset of regurgitation and vomiting in week 2 of life; waves of peristalsis are visible on the abdomen and there is a palpable oval abdominal mass. Treatment is surgical.

Clinical Correlate

Pyloric stenosis is congenital hypertrophy of the pylorus, which presents with projectile vomiting and a palpable abdominal “olive.”

Congenital diaphragmatic hernia occurs when a congenital defect is present in the diaphragm, resulting in herniation of the abdominal organs into the thoracic cavity. The stomach is the most commonly herniated organ due to left-sided congenital

diaphragmatic hernia. Congenital diaphragmatic hernia is often associated with intestinal malrotation. It may be complicated by significant lung hypoplasia.

Hypertrophic Gastropathy

Ménétrier disease is a rare disease of middle-aged men. It is caused by profound hyperplasia of surface mucous cells, accompanied by glandular atrophy. It is characterized by enlarged rugal folds in the body and fundus; clinically, patients experience decreased acid production, protein-losing enteropathy, and increased risk of gastric cancer.

Zollinger-Ellison syndrome. See discussion of gastrinoma in the Pancreas chapter.

Acute Inflammation and Stress Ulcers

Acute hemorrhagic gastritis causes acute inflammation, erosion, and hemorrhage of the gastric mucosa, secondary to a breakdown of the mucosal barrier and acid-induced injury. The etiology is diverse, with initiating agents including chronic aspirin or NSAID use, alcohol use, smoking, recent surgery, burns, ischemia, stress, uremia, and chemotherapy. Patients present with epigastric abdominal pain, or with gastric hemorrhage, hematemesis, and melena.

Gastric stress ulcers are multiple, small, round, superficial ulcers of the stomach and duodenum. Predisposing factors include:

- NSAID use
- Severe stress
- Sepsis
- Shock
- Severe burn or trauma
- Elevated intracranial pressure (Cushing ulcers)

ICU patients have a high incidence of gastric stress ulcer. These ulcers may be complicated by bleeding.

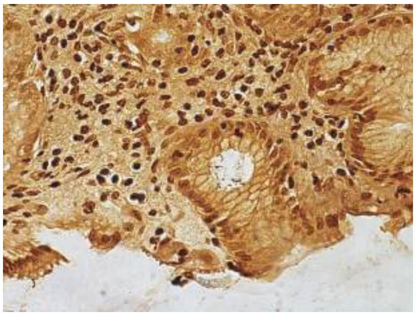
Chronic Gastritis

Chronic gastritis is chronic inflammation of the gastric mucosa, eventually leading to atrophy (chronic atrophic gastritis).

Fundic type chronic gastritis is an autoimmune atrophic gastritis that involves the body and the fundus. It is caused by autoantibodies directed against parietal cells and/or intrinsic factor. The result is loss of parietal cells, decreased acid secretion, increased serum gastrin (G-cell hyperplasia), and pernicious anemia (megaloblastic anemia due to lack of intrinsic factor and B12 malabsorption). Women are affected more than men.

Grossly, one sees a loss of rugal folds in the body and fundus. Microscopically, mucosal atrophy is seen with loss of glands and parietal cells, chronic lymphoplasmacytic inflammation, and intestinal metaplasia. Patients are at increased risk for gastric carcinoma.

Antral type chronic gastritis (also called *Helicobacter pylori* gastritis) is the most common form of chronic gastritis in the United States. The *H. pylori* organisms



Helicobacter pylori, Warthin-Starry stain.
Bradley Gibson, M.D.
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Clinical Correlate

The ability of *H. pylori* to produce urease is clinically used for detection by the [^{13}C]-urea breath test and clofazimine (CLO) tests. Other methods of detection include biopsy (histologic identification is the gold standard) and serology.

are curved, gram-negative rods which produce urease. The risk of infection increases with age. Infection is also associated with duodenal/gastric peptic ulcer, and gastric carcinoma with intestinal type histology.

Microscopically, *H. pylori* organisms are visible in the mucous layer of the surface epithelium. Other microscopic features include foci of acute inflammation, chronic inflammation with lymphoid follicles, and intestinal metaplasia.

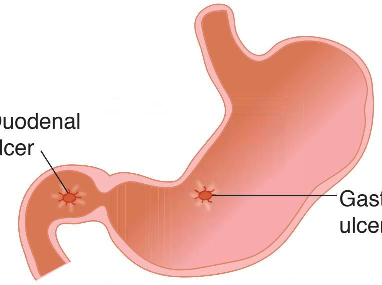
Chronic Peptic Ulcer (Benign Ulcer)

Peptic ulcers are ulcers of the distal stomach and proximal duodenum caused by gastric secretions (hydrochloric acid and pepsin) and impaired mucosal defenses. Predisposing factors include the following:

- Chronic NSAID and aspirin use
- Steroid use
- Smoking
- *H. pylori* infection

Patients present with burning epigastric pain. Diagnosis is by endoscopy with or without biopsy. Treatment is acid suppression (H2 blocker, proton pump inhibitor, etc.) and eradication of *H. pylori*.

Complications of peptic ulcer include hemorrhage, iron deficiency anemia, penetration into adjacent organs, perforation (x-ray shows free air under the diaphragm), and pyloric obstruction.



Duodenal peptic ulcers are more common than gastric peptic ulcers. Associations include the following:

- *H. pylori* (~100%)
- Increased gastric acid secretion
- Increased rate of gastric emptying
- Blood group O
- Multiple endocrine neoplasia (MEN) type I
- Zollinger-Ellison syndrome
- Cirrhosis
- Chronic obstructive pulmonary disease

Most duodenal peptic ulcers are located in the anterior wall of the proximal duodenum.

Gastric peptic ulcers are associated with *H. pylori* (75%). Most are located in the lesser curvature of the antrum. Grossly, they are small (<3 cm), sharply demarcated ('punched out'), solitary with round/oval shape, smooth borders, and radiating mucosal folds.

Gastric Carcinoma (Malignant Ulcer)

Gastric carcinoma is more common in Japan than in the United States, and has a decreasing incidence in the United States. Dietary factors can be risk factors:

- Smoked fish and meats

- Pickled vegetables
- Nitrosamines
- Benzpyrene
- Reduced intake of fruits and vegetables

Other risk factors include *H. pylori* infection, chronic atrophic gastritis, smoking, blood type A, bacterial overgrowth in the stomach, prior subtotal gastrectomy, and Ménétrier disease.

Gastric carcinoma is often (90%) asymptomatic until late in the course, when it can produce weight loss and anorexia. It can also present with epigastric abdominal pain mimicking a peptic ulcer, early satiety, and occult bleeding with iron deficiency anemia.

Most gastric carcinomas are located in the lesser curvature of the antrum. They are large (>3 cm) ulcers with heaped-up margins and a necrotic ulcer base. They may also occur as a flat or polypoid mass. Several histological types occur.

- The **intestinal type** shows gland-forming adenocarcinoma microscopically.
- The **diffuse type** shows diffuse infiltration of stomach by poorly differentiated tumor cells, numerous signet-ring cells (whose nuclei are displaced to the periphery by intracellular mucin), and *linitis plastica* (thickened "leather bottle"–like stomach) gross appearance.

Gastric carcinoma may specifically metastasize to the left supraclavicular lymph node (Virchow sentinel node) and to the ovary (Krukenberg tumor).

Diagnosis is by endoscopy with biopsy; treatment is gastrectomy. The prognosis is poor, with overall 5-year survival ~30%.

GASTRIC LYMPHOMA

Marginal zone B-cell lymphoma and diffuse large B-cell lymphoma occur in the stomach.

SMALL AND LARGE INTESTINES

Mechanical Obstruction

Volvulus is a twisting of a segment of bowel on its vascular mesentery, causing intestinal obstruction and infarction. It is often associated with congenital abnormalities such as intestinal malrotation. Common locations include the sigmoid colon and small bowel; complications include infarction and peritonitis.

Intussusception is the telescoping of a proximal segment of the bowel into the distal segment. It is most common in infants and children. Children present with vomiting, abdominal pain, passage of blood per rectum, and lethargy; a sausage-shaped mass is often palpable in the right hypochondrium.

In adults, intussusception may be caused by a mass or tumor. The intussuscepted segment can become infarcted.

Incarcerated hernia is a segment of bowel that is imprisoned within a hernia; the condition can become complicated by intestinal obstruction and infarction.

Note

Acquired megacolon may be caused by Chagas disease or ulcerative colitis (toxic megacolon).

Bridge to Anatomy

Auerbach plexus = myenteric ganglia

Meissner plexus = submucosal ganglia

Hirschsprung disease (or congenital aganglionic megacolon) is caused by congenital absence of ganglion cells in the rectum and sigmoid colon, resulting in intestinal obstruction. The condition affects males more than females, and can be associated with Down syndrome. Hirschsprung may present with delayed passage of meconium, or with constipation, abdominal distention, and vomiting.

Grossly, the affected segment is narrowed, and there is dilation proximal to the narrow segment (megacolon). Microscopically, there is an absence of ganglion cells in Auerbach and Meissner plexuses, and the diagnosis is established when rectal biopsy demonstrates the absence of ganglion cells. Treatment is resection of the affected segment.

Malabsorption Syndromes

Celiac sprue (or gluten-sensitive enteropathy and nontropical sprue) is caused by hypersensitivity to gluten (and gliadin), resulting in loss of small bowel villi and malabsorption. HLA-DQ2 and/or -DQ8 are carried by most patients. Microscopic exam demonstrates a loss of villi, with increased intraepithelial lymphocytes and increased plasma cells in the lamina propria.

Clinically, it often presents in childhood with malabsorption. Symptoms include abdominal distention, bloating, and flatulence, along with diarrhea, steatorrhea, and weight loss. Dermatitis herpetiformis may occur age >20. In adults, celiac presents between decades 4-7. Treatment is dietary restriction of gluten. There is an increased risk of gastrointestinal cancer.

wikimedia.org.

Figure 16-1. Celiac Disease

Environmental enteropathy (previously known as **tropical sprue**) is a malabsorption disease of unknown etiology (infection and/or nutritional deficiency). It affects residents of low-income countries. Biopsy shows blunting of villi and a lymphocytic infiltrate.

Whipple disease is a rare infectious disease involving many organs, including small intestines, joints, lung, heart, liver, spleen, and central nervous system. It typically affects Caucasian males age 30-50.

The infecting organism is *Tropheryma whipplei*. Microscopically, the small bowel lamina propria is filled with macrophages stuffed with the PAS-positive, gram-positive, rod-shaped bacilli. Patients present with malabsorption, weight loss, and diarrhea. Treatment is antibiotics.

Inflammatory bowel disease (IBD). There are 2 categories of IBD:

- **Crohn's disease (CD)** (or regional enteritis)
- **Ulcerative colitis (UC)**

Colitis of indeterminate type describes cases that cannot be clearly classified.

Caucasians develop IBD more frequently than non-Caucasians. The incidence of IBD is increasing.

Age distribution varies with the disease:

- CD has a bimodal distribution with peaks at age 10–30 and 50–70
- UC peaks at age 20–30

IBD can present with episodes of bloody diarrhea or stools with mucus, crampy lower abdominal pain, or fever. CD may present with malabsorption or extraintestinal manifestations. It may mimic appendicitis. CD may cause perianal fistulas.

Diagnosis of IBD requires endoscopic biopsy and clinicopathologic correlation.

New studies indicate that risk of colorectal carcinoma (CRC) in CD and UC are equivalent for similar extent and duration of disease; the risk of CRC is not as high as previous studies suggested.

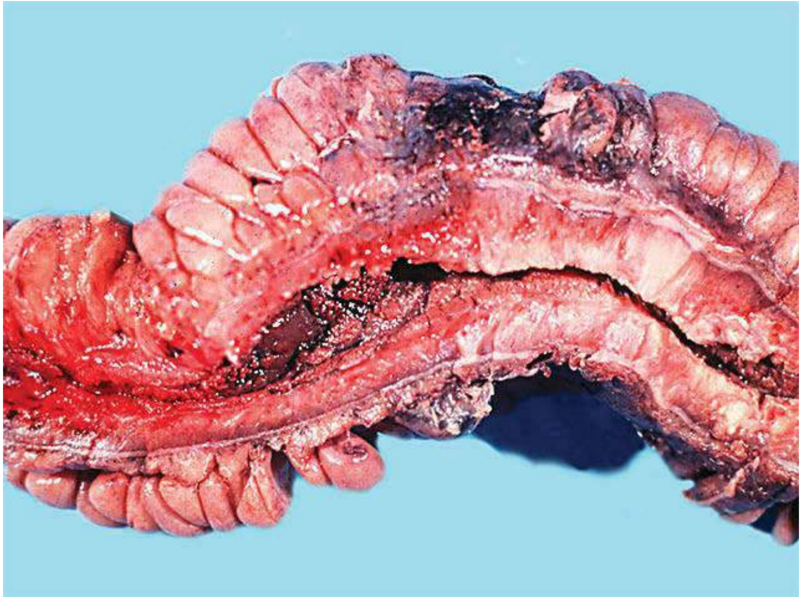


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Figure 16-2. Narrowed Colon Segment in Crohn's Disease

Note

Damage to the ileal mucosa can cause deficiencies of vitamin B12 and folate.

Table 16-1. Crohn's Disease Versus Ulcerative Colitis

	Crohn's Disease	Ulcerative Colitis
Most common site	Terminal ileum	Rectum
Distribution	Mouth to anus	Rectum → colon "back-wash" ileitis
Spread	Discontinuous/"skip"	Continuous
Gross features	• Focal aphthous ulcers with intervening normal mucosa • Linear fissures • Cobblestone appearance • Thickened bowel wall • "Creeping fat"	Extensive ulceration Pseudopolyps
Micro	Noncaseating granulomas	Crypt abscesses
Inflammation	Transmural	Limited to mucosa and submucosa
Complications	• Strictures • "String sign" on barium studies • Obstruction • Abscesses • Fistulas • Sinus tracts	Toxic megacolon
Genetic association		HLA-B27
Extraintestinal manifestations	Common (e.g., migratory polyarthritis, ankylosing spondylitis, primary sclerosing cholangitis, erythema nodosum, pyoderma gangrenosum, uveitis)	Common (e.g., migratory arthritis, ankylosing spondylitis, primary sclerosing cholangitis, erythema nodosum, pyoderma gangrenosum, uveitis)

Miscellaneous Conditions

Ischemic bowel disease is caused by decreased blood flow and ischemia of the bowel, secondary to atherosclerosis with thrombosis, thromboembolism, or reduced cardiac output from shock. It is most common in older individuals. Typical presentation is with abdominal pain and bloody diarrhea. The disease distribution tends to involve watershed areas (e.g., splenic flexure), and affected areas typically show hemorrhagic infarction.

Bridge to Anatomy

The splenic flexure of the colon receives blood from both the superior and inferior mesenteric arteries.

Treatment is surgical resection, but the prognosis is poor, with >50% mortality.

Hemorrhoids are tortuous, dilated anal submucosal veins caused by increased venous pressure. Risk factors include constipation and prolonged straining

during bowel movements, pregnancy, and cirrhosis. Complications include painful thrombosis and streaks of bright red blood on hard stool.

Angiodysplasia is arteriovenous malformations of the intestines. It occurs in the cecum and right colon. Individuals age >55 are most commonly affected, presenting with multiple episodes of rectal bleeding. It is associated with Osler-Weber-Rendu and CREST syndromes. Treatment is surgical resection.

Melanosis coli is common with laxative abuse; it causes black pigmentation of the colon due to the ingestion of the laxative pigment by macrophages in the mucosal and submucosa. It can mimic colitis or malignancy.

Pseudomembranous colitis (antibiotic-associated colitis) is an acute colitis characterized by the formation of inflammatory pseudomembranes in the intestines. It is usually caused by *Clostridium difficile* infection (often brought on by a course of broad-spectrum antibiotics, especially clindamycin and ampicillin), but it can be caused by ischemic bowel disease.

Gross examination shows yellow-tan mucosal membranes. Microscopic exam shows the pseudomembranes are composed of an adherent layer of acute inflammatory cells, mucus and necrotic debris overlying sites of colonic mucosal injury. Presentation is with diarrhea, fever, and abdominal cramps. Diagnosis is established with detection of *C. difficile* toxin in the stool. Treatment of clostridial pseudomembranous colitis is vancomycin or metronidazole.

Appendicitis is most commonly caused by obstruction of the appendix by a fecalith. It often starts with periumbilical pain that subsequently localizes to the right lower quadrant. Nausea, vomiting, and fever may also be present. Lab studies show an elevated white blood cell count. A complication is appendiceal rupture leading to peritonitis. Grossly, a fibrinopurulent exudate may be seen on the appendiceal serosa; microscopically, neutrophils are present within the mucosa and muscular wall (muscularis propria) of the appendix.

Note

Osler-Weber-Rendu Syndrome

- a.k.a. hereditary hemorrhagic telangiectasia
- Autosomal dominant
- Telangiectasias of skin and mucous membranes
- Common on lips, tongue, and fingertips
- May develop iron deficiency anemia

Diverticula

Meckel diverticulum is a congenital small bowel diverticulum caused by persistence of a remnant of the vitelline (omphalomesenteric) duct (*see* Anatomy Lecture Notes). With Meckel, the "rule of 2s" applies:

- 2% of the normal population
- 2 feet from the ileocecal valve
- Length 2 cm
- Age ≤2 years at time of diagnosis

Most Meckel diverticula are asymptomatic but they may contain rests of ectopic gastric mucosa and present with intestinal bleeding.

Colonic diverticulosis is an acquired outpouching of the bowel wall, characterized by herniation of the mucosa and submucosa through the muscularis propria (pseudodiverticulum). It is extremely common in the United States.

- Incidence increases with age
- Major risk factor is a low-fiber diet, which leads to increased intraluminal pressure
- Most common location is sigmoid colon

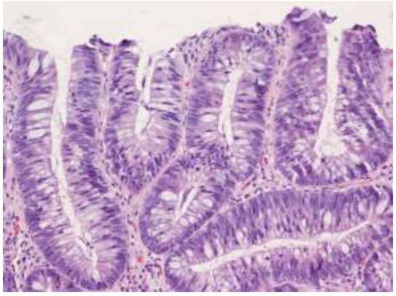
Note

Given that only 2 layers of the bowel wall are involved, these acquired outpouchings are technically pseudodiverticula.

Many cases are asymptomatic and picked up on screening colonoscopy. When symptomatic, it can cause constipation alternating with diarrhea, left lower quadrant abdominal cramping and discomfort, occult bleeding and an iron deficiency anemia, or lower gastrointestinal tract hemorrhage. Complications include diverticulitis, fistulas, and perforation with accompanying peritonitis.

Polyps

Hamartomatous polyps include nonfamilial juvenile polyps and polyps associated with a familial (Peutz-Jeghers) syndrome. Nonsyndromic polyps do not have malignant potential.



Tubular Adenoma
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Hyperplastic polyps are the most common histologic type; they occur most often in the left colon and are usually <5 mm. Although previously considered not to have malignant potential, newer studies suggest they are part of a group of polyps with serrated histology and risk of progression to cancer. **Serrated polyps** occur more often in the right colon.

Tubular and villous adenomas have long been known to have malignant potential. Microscopically, they show cellular dysplasia and either pure tubular, pure villous or tubulovillous histology.

Familial Syndromes

Familial adenomatous polyposis (FAP), also called adenomatous polyposis coli (APC), is due to an autosomal dominant mutation of the *APC* gene on chromosome 5q21.

Affected individuals may develop **thousands of colonic adenomatous polyps**; the diagnosis is made with discovery of >100 adenomatous polyps on endoscopy. **Complications:** by age 40, virtually 100% will develop an invasive adenocarcinoma and increased risks for developing duodenal adenocarcinoma and adenocarcinoma of the papilla of Vater.

Gardner syndrome is an autosomal dominant variant of familial adenomatous polyposis characterized by numerous colonic adenomatous polyps, multiple osteomas, fibromatosis, and epidermal inclusion cysts.

Turcot syndrome is a rare variant of familial adenomatous polyposis characterized by numerous colonic adenomatous polyps and central nervous system tumors (gliomas).

Hereditary nonpolyposis colorectal cancer (HNPCC), or Lynch syndrome, is due to an autosomal dominant mutation of a DNA nucleotide mismatch repair gene that predisposes for colon cancer. It is associated with an increased risk of cancer at other sites, including the endometrium and the ovary.

Peutz-Jeghers syndrome is an autosomal dominant condition characterized by multiple hamartomatous polyps (primarily in the small intestine); melanin pigmentation of the oral mucosa; and increased risk of cancer at numerous sites including the lung, pancreas, breast, and uterus.

Neoplasia

Colonic adenocarcinoma is the third most common tumor in the United States, in terms of incidence and mortality. Risk factors include:

- Dietary factors (low fiber, low fruits/vegetables and high in red meat and animal fat)
- Colon polyps (isolated adenomatous polyps, hereditary polyposis syndromes)
- Other colon disease (Lynch syndrome, ulcerative colitis)

Diagnosis is established via endoscopy with biopsy.

Cancer genetics: Mutations of the *APC* gene cause activation of the Wnt pathway, leading β-catenin to translocate to the nucleus where it causes the overexpression of growth-promoting genes. DNA mismatch repair causes microsatellite instability, which is another genetic carcinogenesis pathway.

The pattern of spread in colonic adenocarcinoma includes lymphatic spread to mesenteric lymph nodes, with distant spread to liver, lungs, and bone. Staging is with the TNM system. Treatment can include surgical resection and chemotherapy (for metastatic disease); CEA levels can be used to monitor for disease recurrence.

Screening is recommended for the general population beginning age 50. Current guidelines suggest:

- Colonoscopy every 10 years or annual fecal occult blood test (FOBT), or
- Combination of FOBT (every 3 years) and sigmoidoscopy (every 5 years)

Note

TNM Staging of Colorectal Cancer

Stage I (T1-2N0M0): tumors that do not penetrate through mucosa (T1) or muscularis (T2)

Stage II (T3N0M0): tumors that have penetrated through the muscularis but have not spread to the lymph nodes

Stage III (TXN1M0): regional lymph node involvement

Stage IV (TXNXM1): metastasis to distant sites

Table 16-2. Right-Sided Cancer Versus Left-Sided Cancer

	Right-Sided Cancer	Left-Sided Cancer
Gross	Polypoid mass	Circumferential growth producing a “napkin-ring” configuration
Barium studies	Polypoid mass	“Apple-core” lesion
Presentation	Bleeding • Occult blood in stool • Iron deficiency anemia	Change in bowel habits • Constipation or diarrhea • Reduced caliber stools • Obstruction

Carcinoid tumors are neuroendocrine tumors that often produce serotonin. Locations include the appendix (most common) and the terminal ileum. Metastasis to the liver may result in carcinoid heart disease.

Note

Histologically, carcinoid tumors appear similar to other neuroendocrine tumors, with nests of small uniform cells.

Carcinoid syndrome is characterized by diarrhea, cutaneous flushing, bronchospasm and wheezing, and fibrosis. The diagnosis is substantiated by demonstrating elevated urinary 5-HIAA (5-hydroxyindoleacetic acid).

Bridge to Biochemistry

Serotonin is converted to 5-HIAA by monoamine oxidase.

Gastrointestinal stromal tumor (GIST) is the most common sarcoma of the GI tract. Most cases have a *KIT* mutation. The peak incidence is in decade 7. Treatment is resection and a tyrosine-kinase inhibitor.

Pancreatic Pathology 17

Learning Objectives

- ❑ Demonstrate understanding of congenital anomalies of the pancreas
- ❑ Use knowledge of inflammation of the pancreas or tumors of the pancreas to solve problems

CONGENITAL ANOMALIES OF THE PANCREAS

- **Pancreatic agenesis** is incompatible with life.
- **Pancreatic divisum** is a variant of pancreatic duct anatomy.
- **Annular pancreas** encircles the duodenum and presents as obstruction.
- **Ectopic pancreatic tissue** can hemorrhage, become inflamed, or give rise to a neuroendocrine tumor. These most often arise in the stomach, duodenum, or jejunum.

INFLAMMATION OF THE PANCREAS

Acute pancreatitis is acute inflammation caused by injury to the exocrine portion of the pancreas. The etiology is diverse:

- Gallstones
- Alcohol
- Hypercalcemia
- Drugs
- Shock
- Infections
- Trauma
- Scorpion stings

Pancreatic acinar cell injury results in activation of pancreatic enzymes and enzymatic destruction of the pancreatic parenchyma.

Symptoms include stabbing epigastric abdominal pain radiating to the back. Severe acute pancreatitis can also cause shock. Lab studies show elevated serum amylase and lipase. Complications include acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), pancreatic pseudocyst; pancreatic calcifications, and hypocalcemia. Severe cases have a 30% mortality rate.

Note

Pancreatic pseudocyst is a fluid-filled sac adjacent to the pancreas. The wall of granulation tissue lacks an epithelial lining.

- Gross pathologic examination shows focal hemorrhage and liquefication in the pancreas, accompanied by chalky, white-yellow fat necrosis of adjacent adipose tissue.
- Microscopically there is liquefactive necrosis of the pancreatic parenchyma with acute inflammation and enzymatic fat necrosis.
- Necrosis of blood vessels causes hemorrhage.

Chronic pancreatitis refers to irreversible changes in pancreatic function with accompanying chronic inflammation, atrophy, and fibrosis of the pancreas secondary to repeated bouts of pancreatitis. Manifestations include abdominal pain, pancreatic insufficiency and malabsorption, pancreatic calcifications, pseudocyst, and secondary diabetes mellitus (late complication).

It is common in middle-aged male alcoholics. Pathology shows grossly firm, white, and fibrotic pancreas. Microscopically there is extensive fibrosis with parenchymal atrophy and chronic inflammation.

Autoimmune pancreatitis can occur in association with IgG4-associated fibrosing disorders; this variant responds to steroid therapy.

PANCREATIC TUMORS

Pancreatic neuroendocrine tumors (islet cell tumors) are less common than exocrine tumors. Most are considered low-grade malignancies. Some patients lack laboratory evidence of hormone overproduction. These tumors are not distinguishable from each other on the basis of gross appearance or histology.

- **Insulinoma (β-cell tumor)** (most common type of islet cell tumor)
  - Produces insulin
  - Can cause hypoglycemia, sweating, hunger, confusion, and insulin coma
  - Surgical excision is curative
- **Gastrinoma (G-cell tumor)**
  - Produces gastrin
  - Excess gastrin manifests as Zollinger-Ellison syndrome, which is characterized by thick gastric folds, elevated serum gastrin, gastric hyperacidity, and intractable peptic ulcers
  - Gastrinomas may arise outside the pancreas
  - May be associated with MEN I
- **Glucagonoma** (α-cell tumor)
  - Produces glucagon
  - Excess glucagon causes hyperglycemia (diabetes), anemia, and skin rash
- **Somatostatinoma** (δ-cell tumor)
  - Produces somatostatin
  - Excess somatostatin inhibits insulin secretion, leading to diabetes
  - Can also inhibit gastrin secretion (leading to hypochlorhydria) and cholecystokinin secretion (leading to gallstones and steatorrhea)
  - Prognosis is poor

- **VIPoma**
  - Produces vasoactive intestinal peptide (VIP)
  - Excess VIP causes WDHA syndrome: watery diarrhea, hypokalemia, and achlorhydria

Pancreatic carcinoma is the third most common cause of cancer death in the United States.

- Most common ages 60-80
- Smoking is a risk factor
- Presents with only vague signs and symptoms until late in course
- When more definitive signs and symptoms develop, they can include abdominal pain, migratory thrombophlebitis, and obstructive jaundice

The tumor may occur in the head (60%), body (15%), and tail (5%). Microscopically, the adenocarcinoma arises from the duct epithelium. Tumor desmoplasia and perineural invasion are common. Tumor markers for pancreatic carcinoma include CEA and CA19-9, but they are not useful screening assays.

Treatment is surgical excision (Whipple procedure). The prognosis is very poor, with 5-year survival only ~5%.

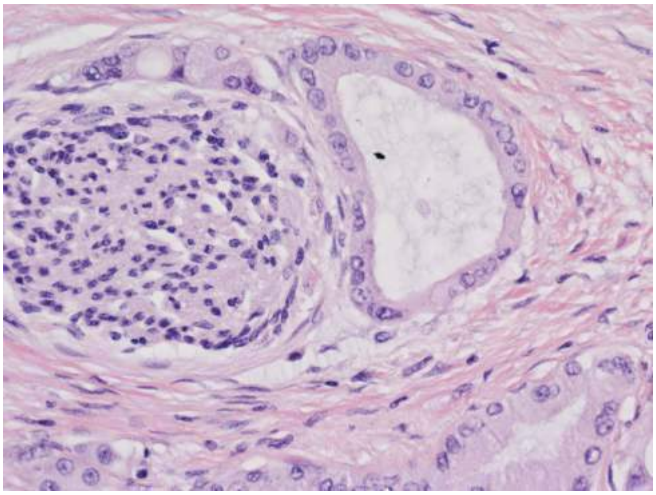

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Figure 17-1. Pancreatic Adenocarcinoma with Perineural Invasion

Pancreatic cystic neoplasms: Serous neoplasms account for 25% of pancreatic cystic neoplasms; most are benign (cystadenomas) and the tumors carry a mutation of *VHL*.

Mucinous neoplasms: Mucinous cystic neoplasms are common in women and can harbor dysplasia or carcinoma; distal pancreatectomy is curative in most cases. **Intraductal papillary mucinous neoplasms** are common in men and tend to arise in the head of the pancreas; *GNAS* mutations are common and carcinoma may arise in the neoplasm.

Gallbladder and Biliary Tract Pathology 18

Learning Objectives

- ❑ Use knowledge of gallstones (cholelithiasis), inflammatory conditions of the gallbladder, and miscellaneous conditions to solve problems
- ❑ Explain information related to biliary tract cancer

GALLSTONES (CHOLELITHIASIS)

Gallstones are frequently asymptomatic but can cause biliary colic (right upper quadrant pain due to impacted stones). Diagnosis is by U/S; the majority of stones are not radiopaque. Complications include cholecystitis, choledocholithiasis (calculi within the biliary tract), biliary tract obstruction, pancreatitis, and cholangitis.

Cholesterol stones are composed mostly of cholesterol monohydrate. The incidence increases with age. Risk factors include female gender, obesity, pregnancy, oral contraceptives, and hormone replacement therapy. Native American Pima and Navajo Indians have an increased incidence of cholesterol gallstones.

Pigmented bilirubinate stones are composed of calcium salts and unconjugated bilirubin. Risk factors are chronic hemolytic anemias, cirrhosis, bacterial infection, and parasites (*Ascaris* or *Clonorchis* [*Opisthorchis*] *sinensis*).

Note

Formation of cholesterol stones involves the precipitation of cholesterol from supersaturated bile.

Clinical Correlate

Murphy's sign is inspiratory arrest in response to palpation of the right subcostal area during deep inspiration. It is seen in patients with pain due to cholecystitis.

INFLAMMATORY CONDITIONS

Acute cholecystitis is an acute inflammation of the gallbladder, usually caused by cystic duct obstruction by gallstones. It can present with biliary colic, right upper quadrant tenderness on palpation, nausea and vomiting, low-grade fever, and leukocytosis. Complications include gangrene of the gallbladder, perforation and peritonitis, fistula formation and gallstone ileus (small bowel obstruction by a large gallstone). Acute acalculous cholecystitis is associated with surgery, trauma, and sepsis.

Chronic cholecystitis is ongoing chronic inflammation of the gallbladder, usually caused by gallstones. Well-developed examples show stromal and mural lymphocytic and plasmacytic infiltrates. Macrophages and granulomas may also be present. The wall is thickened.

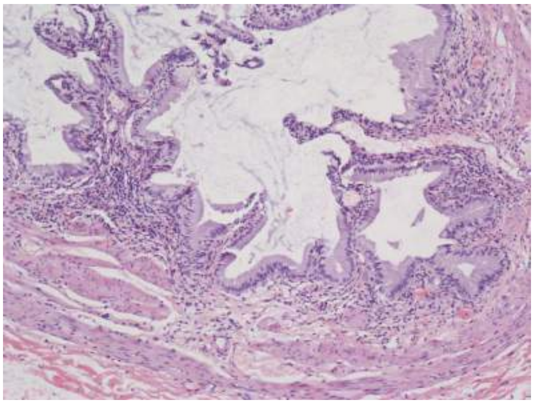

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Figure 18-1. Chronic Cholecystitis

Clinical Correlate

Charcot's triad is RUQ pain, jaundice, and fever, characteristic of acute cholangitis.

Ascending cholangitis is a bacterial infection of the bile ducts ascending up to the liver, usually associated with obstruction of bile flow oftentimes from bile duct stones. It presents with biliary colic, jaundice, high fever, and chills. The infecting organisms are usually gram-negative enteric bacteria.

MISCELLANEOUS CONDITIONS

Cholesterolosis refers to an accumulation of cholesterol-laden macrophages within the mucosa of the gallbladder wall. Gross examination shows yellow speckling of the red-tan mucosa ("strawberry gallbladder"). Microscopic examination shows lipid-laden macrophages within the lamina propria.

Hydrops of the gallbladder (mucocele) occurs when chronic obstruction of the cystic duct leads to the resorption of the normal gallbladder contents and enlargement of the gallbladder by the production of large amounts of clear fluid (hydrops) or mucous secretions (mucocele).

BILIARY TRACT CANCER

Clinical Correlate

Courvoisier law is a palpable gallbladder more likely to be caused by obstruction due to malignancy than by stones.

"Porcelain gallbladder" is a calcification of the gallbladder due to chronic inflammation; recent studies have cast doubt on its association with carcinoma.

Gallbladder cancer is frequently asymptomatic until late in the course. When the tumor does present, it may be with cholecystitis, enlarged palpable gallbladder, or biliary tract obstruction (uncommon). X-ray may show a calcified "porcelain gallbladder." Microscopically, the tissues show adenocarcinoma. The prognosis for gallbladder cancer is poor; 5-year survival rate is ~12%.

Bile duct cancer. Bile duct carcinoma is carcinoma of the **extrahepatic** bile ducts, while cholangiocarcinoma is carcinoma of the **intrahepatic** bile ducts. Klatskin tumor is a carcinoma of the bifurcation of the right and left hepatic bile ducts. Risk factors for bile duct cancer include *Clonorchis (Opisthorchis) sinensis* (liver fluke) in Asia and primary sclerosing cholangitis. Bile duct cancer typically presents with biliary tract obstruction. Microscopic examination shows adenocarcinoma arising from the bile duct epithelium. The prognosis is poor.

Adenocarcinoma of the ampulla of Vater may exhibit duodenal, biliary, or pancreatic epithelium. Patients present with painless jaundice. The 5-year survival rate is <50% in spite of resection.

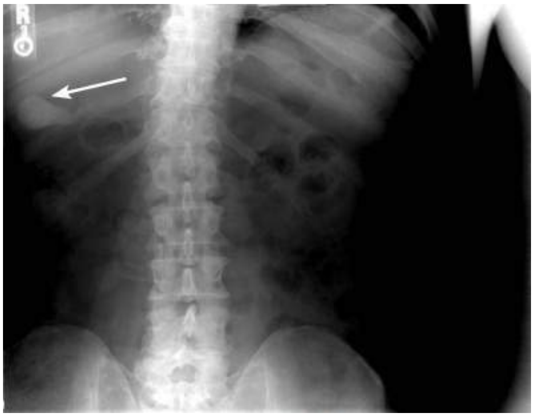

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Figure 18-2. X-Ray Showing Calcified (Porcelain) Gallbladder

Liver Pathology 19

Learning Objectives

- ❑ Solve problems concerning jaundice, cirrhosis, viral hepatitis, amebic liver abscess, and alcoholic liver disease
- ❑ Differentiate metabolic liver disease from hemodynamic liver disease
- ❑ Solve problems concerning liver tumors

LIVER DYSFUNCTION

Dysfunction of the liver may be divided into 4 categories that may coexist:

- **Hepatic failure** occurs in the setting of hepatic necrosis secondary to acute liver failure, chronic liver disease, and hepatocyte dysfunction.
- **Portal hypertension** occurs in the setting of cirrhosis or increased portal venous blood flow.
- **Cholestasis** occurs in the setting of impaired bile flow due to hepatocyte dysfunction or biliary obstruction.
- **Cirrhosis** occurs in the setting of hepatocyte injury and is usually an irreversible nodular regeneration that is end stage.

JAUNDICE

Clinical jaundice occurs with bilirubin levels >2–3 mg/dL. The classic presentation is yellow skin (jaundice) and sclera (icterus). Causes of jaundice include overproduction of bilirubin, defective hepatic bilirubin uptake, defective conjugation, and defective excretion.

Table 19-1. Unconjugated Versus Conjugated Bilirubinemia

Unconjugated (Indirect) Bilirubinemia	Conjugated (Direct) Bilirubinemia
Increased RBC turnover (hemolytic anemias)	Biliary tract obstruction
Physiologic (newborn babies)	Biliary tract disease (PSC and PBC)
Hereditary (Gilbert and Crigler-Najjar syndromes)	Hereditary (Dubin-Johnson and Rotor syndromes)
	Liver disease (cirrhosis and hepatitis)

Clinical Correlate

In infants, increased levels of unconjugated bilirubin (lipid-soluble) may cross the blood–brain barrier and deposit in the basal ganglia, causing irreversible brain damage (kernicterus).

Increased red blood cell (RBC) turnover. RBCs are the major source of bilirubin. Jaundice related to overproduction of bilirubin can be seen in hemolytic anemia and ineffective erythropoiesis (thalassemia, megaloblastic anemia, etc.). Laboratory studies show increased unconjugated bilirubin. Chronic hemolytic anemia patients often develop pigmented bilirubinate gallstones. The most common cause of marked jaundice in the newborn is blood group incompatibility (most commonly ABO) between mother and child, causing **hemolytic disease of the newborn**.

Physiologic jaundice of the newborn is a transient unconjugated hyperbilirubinemia due to the immaturity of the liver. Risk factors include prematurity and hemolytic disease of the newborn (erythroblastosis fetalis). Physiologic jaundice of the newborn can be complicated by kernicterus; treatment is phototherapy. Jaundice also occurs in newborns who have infections.

Hereditary hyperbilirubinemias

When hyperbilirubinemia is prolonged in the newborn, a mutation affecting bilirubin conjugation enters the differential diagnosis.

- **Gilbert syndrome** is a common benign inherited disorder that causes unconjugated hyperbilirubinemia due to bilirubin UDP-glucuronosyltransferase (UGT) deficiency. Kernicterus rarely occurs and the treatment is phenobarbital.
- **Crigler-Najjar syndrome** causes unconjugated hyperbilirubinemia due to bilirubin glucuronosyltransferase (UGT) absence or deficiency. Treatment for type 1 is gene replacement therapy and liver transplantation. For a milder type 2, phenobarbital is used.
- **Dubin-Johnson syndrome** is a benign autosomal recessive disorder characterized by decreased bilirubin excretion due to a defect in the canalicular cationic transport protein. It produces conjugated hyperbilirubinemia and a distinctive black pigmentation of the liver, but has no clinical consequences.
- **Rotor syndrome** is an autosomal recessive conjugated hyperbilirubinemia that is similar to Dubin-Johnson syndrome, but without liver pigmentation. There are no clinical consequences.

Biliary tract obstruction may have multiple etiologies, including gallstones; tumors (pancreatic, gallbladder, and bile duct); stricture; and parasites (liver flukes—*Clonorchis [Opisthorchis] sinensis*). The presentation can include jaundice and icterus; pruritus due to increased plasma levels of bile acids; abdominal pain, fever, and chills; dark urine (bilirubinuria); and pale clay-colored stools. Lab studies show elevated conjugated bilirubin, elevated alkaline phosphatase, and elevated 5′-nucleotidase.

Primary biliary cirrhosis (PBC) is a chronic liver disease that is characterized by inflammation and granulomatous destruction of intrahepatic bile ducts. Females have 10 times the incidence of primary biliary cirrhosis compared to males; the peak incidence is age 40–50.

Presentation includes obstructive jaundice and pruritus; xanthomas, xanthelasmas, and elevated serum cholesterol; fatigue; and cirrhosis (late complication). Most patients have another autoimmune disease (scleroderma, rheumatoid arthritis or systemic lupus erythematosus).

Laboratory studies show elevated conjugated bilirubin, elevated alkaline phosphatase, and elevated 5′-nucleotidase. Treatment with oral ursodeoxycholic acid slows disease progression. **Antimitochondrial autoantibodies** (AMA) are present in >90% of cases, which further supports an autoimmune basis. Microscopically, lymphocytic and granulomatous inflammation involves interlobular bile ducts.

Primary sclerosing cholangitis (PSC) is a chronic liver disease characterized by segmental inflammation and fibrosing destruction of intrahepatic and extrahepatic bile ducts. The exact etiologic mechanism is not known but growing evidence supports an immunologic basis.

The male to female ratio is 2:1; peak age 20–40. Most cases of PSC are associated with ulcerative colitis. The presentation is similar to PBC. Complications include biliary cirrhosis and cholangiocarcinoma.

Microscopically, there is periductal chronic inflammation with concentric fibrosis around bile ducts and segmental stenosis of bile ducts. Cholangiogram shows “beaded appearance” of bile ducts.

CIRRHOSIS

Cirrhosis is end-stage liver disease characterized by disruption of the liver architecture by bands of fibrosis which divide the liver into nodules of regenerating liver parenchyma.

Causes of cirrhosis include alcohol, viral hepatitis, biliary tract disease, hemochromatosis, cryptogenic/idiopathic, Wilson disease, and α-1-antitrypsin deficiency.

On gross Pathology, **micronodular** cirrhosis has nodules <3 mm, while **macronodular** cirrhosis has nodules >3 mm; mixed micronodular and macronodular cirrhosis can also occur. At the end stage, most diseases result in a mixed pattern, and the etiology may not be distinguished based on the appearance.

Cirrhosis has a multitude of **consequences**, including portal hypertension, ascites, splenomegaly/hypersplenism, esophageal varices, hemorrhoids, caput medusa, decreased detoxification, hepatic encephalopathy, spider angiomata, palmar erythema, gynecomastia, decreased synthetic function, hepatorenal syndrome and coagulopathy.

Clinical Correlate

Prothrombin time, not partial thromboplastin time, is used to assess the coagulopathy due to liver disease.

VIRAL HEPATITIS

Viral hepatitis can be asymptomatic or it can present with malaise and weakness, nausea and anorexia, jaundice, or dark urine. Lab studies show markedly elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Diagnosis is by serology.

Acute viral hepatitis is viral hepatitis with signs and symptoms for <6 months. It can be caused by any of the hepatitis viruses.

Microscopically, the liver shows lobular disarray, hepatocyte swelling (balloon cells), apoptotic hepatocytes (Councilman bodies), lymphocytes in portal tracts and in the lobule, hepatocyte regeneration, and cholestasis.

Clinical Correlate

Non-hepatitis viruses which may infect the liver include:

- *Epstein-Barr virus* (EBV)—infectious mononucleosis
- *Cytomegalovirus* (CMV)
- Herpes
- Yellow fever

Clinical Correlate

Hepatitis D requires hepatitis B to propagate.

Chronic viral hepatitis is viral hepatitis with signs and symptoms for >6 months. It can be caused by hepatitis viruses B, C, and D.

- Microscopically, **chronic persistent hepatitis** shows inflammation confined to portal tracts.
- **Chronic active hepatitis** shows inflammation spilling into the parenchyma, causing interface hepatitis (piecemeal necrosis of limiting plate).

Hepatitis B often has “ground glass” hepatocytes (due to cytoplasmic HBsAg).

Table 19-2. The Hepatitis Viruses

Common Virus Name	Hepatitis A (HAV)	Hepatitis B (HBV)	Hepatitis C (HCV)	Hepatitis D (HDV)	Hepatitis E (HEV)
Common disease name	“Infectious”	“Serum”	“Post-transfusion” or “non-A, non-B”	“Delta”	“Enteric”
Virus	*Hepatovirus* nonenveloped capsid RNA	*Hepadnavirus* enveloped DNA	*Flavivirus* enveloped RNA	Defective enveloped circular RNA	*Hepevirus* nonenveloped capsid RNA
Transmission	Fecal-oral	Parenteral, sexual, perinatal	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Severity	Mild	Occasionally severe	Usually subclinical	Co-infection with HBV occasionally severe; super-infection with HBV often severe	Normal patients: mild; pregnant patients: severe
Chronicity or carrier state	No	Yes	Yes (high)	Yes	No
Clinical diseases	Acute hepatitis	• Acute hepatitis • Chronic hepatitis • Cirrhosis • Hepatocellular carcinoma (HCC)	• Chronic hepatitis • Cirrhosis • HCC	• Acute hepatitis • Chronic hepatitis • Cirrhosis • HCC	Acute hepatitis
Laboratory diagnosis	Symptoms and anti-HAV IgM	Symptoms and serum levels of HBsAg, HBeAg, and anti-HBc IgM	Symptoms and EIA for anti-HCV	Anti-HDV ELISA	Tests not routinely available
Prevention	Vaccine, hygiene	Vaccine			Hygiene
Treatment	Supportive	Antivirals, interferons, transplant	Antivirals, interferons, transplant	See hepatitis B	Supportive

Table 19-3. Hepatitis B Terminology and Markers

Abbreviation	Name and Description
HBV	Hepatitis B virus, a hepadnavirus (enveloped, partially double-stranded DNA virus); Dane particle = infectious HBV
HBsAg	Antigen found on surface of HBV; also found on spheres and filaments in patient's blood: positive during acute disease; continued presence indicates carrier state
HBsAb	Antibody to HBsAg; provides immunity to hepatitis B
HBcAg	Antigen associated with core of HBV
HBcAb	Antibody to HBcAg; positive during window phase; IgM HBcAb is an indicator of recent disease
HBeAg	A second, different antigenic determinant on the HBV core; important indicator of transmissibility
HBeAb	Antibody to e antigen; indicates low transmissibility
Delta agent	Small RNA virus with HBsAg envelope; defective virus that replicates only in HBV-infected cells

Table 19-4. Hepatitis A Serology

Acute or recent infection	anti-HAV IgM
Prior infection or immunization	anti-HAV IgG

Table 19-5. Hepatitis B Serology

	HBsAg HBeAg* HBV-DNA	HBcAb IgM	HBcAb IgG	HBsAb IgG
Acute infection	+	+	–	–
Window period	–	+	–	–
Prior infection	–	–	+	+
Immunization	–	–	–	+
Chronic infection	+	–	+	–

*HBeAg—correlates with viral proliferation and infectivity

AMEBIC LIVER ABSCESS

Amebic liver abscess is rare in the United States except in those who have traveled to/from tropical areas with poor sanitation. The causative organism is *Entamoeba histolytica*. The presentation, which may occur years after travel, includes RUQ pain, fever, and hepatic tenderness.

Detection of a space-occupying liver lesion with positive serology is diagnostic. Treatment is antibiotics. Drainage is rarely necessary.

ALCOHOLIC LIVER DISEASE

Fatty change (steatosis) is reversible with abstinence. The gross appearance is of an enlarged, yellow, greasy liver. Microscopically, the liver initially shows centrilobular macrovesicular steatosis (reversible) that can eventually progress to fibrosis around the central vein (irreversible).

Alcoholic hepatitis is an acute illness that usually follows a heavy drinking binge. Some patients have no symptoms and others develop RUQ pain, hepatomegaly, jaundice, malaise, anorexia, or even fulminant liver failure.

Microscopically, the liver shows hepatocyte swelling (ballooning) and necrosis, Mallory bodies (cytokeratin intermediate filaments), neutrophils, fatty change, and eventual fibrosis around the central vein. The prognosis can be poor, since each episode has a 20% risk of death, and repeated episodes increase the risk of developing cirrhosis.

Alcoholic cirrhosis develops in 15% of alcoholics, and is typically a micronodular or Laennec cirrhosis.

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Figure 19-1. Alcoholic Cirrhosis, Liver

METABOLIC LIVER DISEASE

Wilson disease (hepatolenticular degeneration) is a genetic disorder of copper metabolism resulting in the accumulation of toxic levels of copper in various organs. It affects the liver (fatty change, chronic hepatitis, and micronodular cirrhosis), cornea (Kayser-Fleischer rings [copper deposition in Descemet's membrane]), and brain (neurological and psychiatric manifestations, movement disorder).

Diagnosis is established by demonstrating decreased serum ceruloplasmin levels, increased tissue copper levels (liver biopsy), and increased urinary copper excretion. Treatment includes copper chelators (D-penicillamine); liver transplantation is curative.

The disease is autosomal recessive, and the WD gene (*ATP7B* on chromosome 13) codes for a hepatocyte canalicular copper-transporting ATPase. Damage to the gene leads to a decreased biliary excretion of copper. Wilson disease presents in children or adolescents with liver disease.

Hemochromatosis is a disease of increased levels of iron, leading to tissue injury. **Hereditary (primary) hemochromatosis** is a recessive disorder of the *HFE* gene on chromosome 6p. The most common mutation of the *HFE* gene is the C282Y mutation, which increases small intestine absorption of iron. **Secondary hemochromatosis** can follow transfusions for chronic anemias. Hemochromatosis affects 5 times as many males as females, and the disease is common in people of Northern European descent.

Hemochromatosis can cause micronodular cirrhosis and hepatocellular carcinoma (200 times the normal risk ratio); secondary diabetes mellitus; hyperpigmented skin ("bronzing"); congestive heart failure and cardiac arrhythmias; and hypogonadism. Diagnosis is established by demonstrating markedly elevated serum iron and ferritin or increased tissue iron levels (Prussian blue stain) on liver biopsy. Treatment is phlebotomy.

α-1-antitrypsin deficiency is an autosomal recessive disorder characterized by production of defective α-1-antitrypsin (α1-AT), which accumulates in hepatocytes and causes liver damage and low serum levels of α1-AT.

α1-AT is produced by the *SERPINA1* gene (chromosome 14); >75 gene variants are described. Normal individuals are homozygous *PiMM*. Heterozygotes have intermediate levels of the enzyme. Homozygous *PiZZ* have severe reductions (10% of normal) in enzyme levels.

α-1-antitrypsin deficiency affects the liver (micronodular cirrhosis and an increased risk of hepatocellular carcinoma) and lungs (panacinar emphysema). Microscopically, PAS positive, eosinophilic cytoplasmic globules are found in hepatocytes. Treatment includes smoking abstinence/cessation to prevent emphysema; liver transplantation is curative.

Note

Protease-Antiprotease Imbalance

α-1-antitrypsin is an important protease inhibitor.

- Responsible for inhibiting neutrophil elastase
- Inhibits trypsin, chymotrypsin, and bacterial proteases

Reye syndrome is a rare, potentially fatal disease that occurs in young children with viral illness (varicella or influenza) treated with aspirin. The disease mechanism is unknown; mitochondrial injury and dysfunction play an important role. Reye causes hepatic fatty change (microvesicular steatosis) and cerebral edema/encephalopathy. There is complete recovery in 75% of patients, but those that do not recover may experience permanent neurologic deficits. Coma and death may result. Treatment is supportive.

Nonalcoholic fatty liver disease is a disease of lipids accumulating in hepatocytes that is not associated with heavy alcohol use. It occurs equally in men and women, and is strongly associated with obesity, hyperinsulinemia, insulin resistance, and type 2 diabetes mellitus.

The pathogenesis involves lipid accumulation in hepatocytes that can progress to steatohepatitis (NASH—nonalcoholic steatohepatitis) and finally cirrhosis. Nonalcoholic fatty liver disease is a diagnosis of exclusion.

HEMODYNAMIC LIVER DISEASES

Budd-Chiari syndrome (hepatic vein thrombosis) refers to occlusion of the hepatic vein by a thrombus, often resulting in death. While a few cases are idiopathic, more often there is an underlying process predisposing for the thrombosis e.g., polycythemia vera, pregnancy, oral contraceptives, paroxysmal nocturnal hemoglobinuria, or hepatocellular carcinoma. It presents with abdominal pain, hepatomegaly, ascites, jaundice, splenomegaly, and in some cases, death.

The initial diagnostic test is ultrasonography. Microscopically, the liver shows centrilobular congestion and necrosis. In the chronic form, fibrosis develops. Treatment includes supportive care and treatment of the underlying condition. Some patients require lifelong anticoagulation.

Chronic passive congestion of the liver refers to a "backup of blood" into the liver, usually due to right-sided heart failure. Grossly, the liver characteristically has a nutmeg pattern of alternating dark (congested central areas) and light (portal tract areas) liver parenchyma. Microscopically, the liver shows centrilobular congestion.

Complications include centrilobular necrosis, which is an ischemic necrosis of centrilobular hepatocytes. Long-standing congestion can lead to centrilobular fibrosis, which can eventually become cardiac cirrhosis (sclerosis).

LIVER TUMORS

Hemangioma is the most common primary tumor of the liver, mostly affecting women. It is a benign vascular tumor that typically forms a subcapsular, red, spongy mass. It is often asymptomatic and detected incidentally on CT or MRI. Resection is rarely indicated, and liver biopsy carries the risk of bleeding.

Hepatocellular adenoma (HCA) affects young women and is related to oral contraceptive use. Half of cases are asymptomatic. Symptoms include abdominal pain or spontaneous intraperitoneal hemorrhage (25% of cases). Due to the risk of transformation to HCC, resection is often recommended.

There are 3 subtypes of HCA:

- **H-HCA** is a solitary or multiple tan steatotic nodule with rare transformation into HCC
  - Mutation of hepatocyte nuclear factor 1
- **b-HCA** can resemble HCC histologically and transforms to HCC in some cases
  - Was named for the associated beta-catenin mutations
- **I-HCA** shows inflammatory infiltrates, sinusoidal dilatation, and thick-walled arteries
  - Acute inflammatory markers are elevated; malignant transformation occurs less frequently

Focal nodular hyperplasia is subcapsular lesion often discovered incidentally by the radiologist. Laboratory values are generally normal. It is a nodular proliferation in response to a vascular anomaly. There is a central, stellate scar. Excision is generally not required due to the characteristic appearance on imaging.

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver in adults. The incidence is higher in Asia and Japan than in the United States. Risk factors include cirrhosis, hepatitis B and C viruses, alcohol, aflatoxin B1.

HCC has a tendency for hematogenous spread and invasion of portal and hepatic veins. The tumor marker is α-fetoprotein (AFP).

The fibrolamellar variant affects younger age, has fibrous bands, and has a better prognosis.

Angiosarcoma is a rare, fatal tumor associated with exposure to vinyl chloride.

Hepatoblastoma is the most common hepatic malignancy in infants and children. Lobectomy is the standard of care. Histology shows immature precursor cells.

Metastatic tumors are the most common tumors found within the liver. Common primary sites include the colon, breast, and lung. Metastatic tumors tend to occur as multiple well-circumscribed masses.

Central Nervous System Pathology 20

Learning Objectives

- ❑ Solve problems concerning infections across the blood brain barrier
- ❑ Answer questions about cerebrovascular disease, CNS trauma, and brain herniation
- ❑ Use knowledge of developmental abnormalities and perinatal brain injury
- ❑ Explain information related to demyelinating disorders
- ❑ Solve problems concerning degenerative and dementing disorders
- ❑ Describe CNS tumors

INFECTIONS

Meningitis is inflammation of the 2 inner meningeal layers, the pia and the arachnoid.

Acute aseptic (viral) meningitis is caused by leptomeningeal inflammation due to viruses (enterovirus most frequent); the inflammation produces a lymphocytic infiltration of leptomeninges and superficial cortex. Patients present with fever, signs of meningeal irritation, and depressed consciousness. Mortality is low. Viral meningitis carries a better prognosis than bacterial meningitis.

Acute viral meningitis is the most common neurologic symptom associated with primary HIV infection; it presents around the time of seroconversion with an acute confusional state. Symptoms resolve after 1 month with supportive care.

Acute purulent (bacterial) meningitis is a purulent leptomeningeal inflammation.

- *Streptococcus pneumoniae* is the most common cause of meningitis in infants, young children, and adults.
- Neonates are infected most frequently with group B streptococci but *Escherichia coli* causes a greater number of fatalities.
- *Neisseria meningitidis* is seen in teens and young adults and is often associated with a maculopapular rash.
- The incidence of *Listeria monocytogenes* increases after age 50. This pathogen also tends to infect people with poor cell-mediated immunity.

The leptomeninges are opaque on gross examination. Microscopic examination shows neutrophilic infiltration of the leptomeninges, extending variably to cortex. Diffuse cerebral edema carries a risk of fatal herniations. The classic triad of bacterial meningitis is fever, nuchal rigidity, and altered mental status.

Mycobacterial meningoencephalitis can be caused by *Mycobacterium tuberculosis* or atypical mycobacteria. It occurs in patients who have reactivation of latent infection and immunocompromised patients such as AIDS patients (*Mycobacterium avium-intracellulare*). Diagnosis requires microscopy/culture of large volumes of CSF. MRI is the imaging test of choice and shows basal meningeal enhancement and hydrocephalus. It usually involves the basal surface of the brain, and may cause characteristic tuberculomas within the brain and dura mater.

Table 20-1. CSF Parameters in Different Forms of Meningitis

Condition	Cells/µL	Glucose (µg/dL)	Proteins (mg/dL)	Pressure (mm H_2O)
Normal values	<5 lymphocytes	45–85 (50–70% glycemia)	15–45	70–180
Purulent (bacterial)	Up to 90,000 neutrophils	Decreased (<45)	Increased (>50)	Markedly elevated
Aseptic (viral)	100–1,000 most lymphocytes	Normal or decreased	Normal or slightly increased (>50)	Normal or slightly elevated
Granulomatous (mycobacterial/fungal)	100–1,000 most lymphocytes	Decreased (<45)	Increased (>50)	Moderately elevated

The **viral encephalitides** have common features of perivascular cuffs, microglial nodules, neuron loss, and neuronophagia. Clinical manifestations are variable, and can include mental status change, fever, and headache, often progressing to coma.

- Arthropod-borne forms can be due to St. Louis, Eastern and Western equine, and Venezuelan encephalitides.
- Herpes simplex type 1 produces a characteristic **hemorrhagic necrosis of temporal lobes**. Cowdry type A bodies are intranuclear inclusions seen in neurons and glial cells.
- **Rabies** has characteristic Negri bodies in the cytoplasm of hippocampal and Purkinje cells.
- **HIV encephalopathy** shows histopathology of microglial nodules and diagnostic multinucleated giant cells. Spinal involvement leads to vacuolar myelopathy, which is similar to vitamin B12 deficiency–associated subacute combined degeneration.
- **Progressive multifocal leukoencephalopathy (PML)** is caused by JC polyomavirus. It occurs in immunocompromised patients and patients taking immunomodulatory therapies. Neurologic symptoms are varied and include impairment of cognition and motor function. There is no specific antiviral drug and mortality is high. Tissue sections show areas of demyelination and enlarged oligodendrocytes.

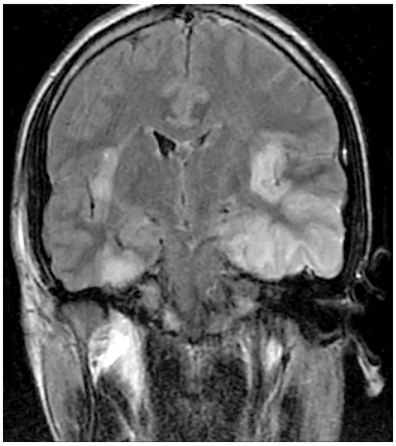

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Figure 20-1. CT Scan Showing Edema of Bilateral Temporal Lobes, Related to Herpes Simplex Encephalitis

Fungal meningoencephalitides. *Candida*, *Aspergillus*, *Cryptococcus*, and *Mucor* species are the most frequent agents. *Aspergillus* and *Mucor* have a marked tropism for blood vessels, which leads to vasculitis, rupture of blood vessels, and hemorrhage. *Cryptococcus* causes diffuse meningoencephalitis, which leads to invasion of the brain through the Virchow-Robin space (a continuation of the subarachnoid space around blood vessels entering the neuropil) and soap bubble lesions.

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*. It is common in AIDS patients, and the condition causes cerebral abscess with central necrosis and chronic inflammation. MRI/CT scan shows a characteristic ring-enhancing lesion.

Cerebral abscess can occur as a result of either hematogenous dissemination or direct spread from contiguous foci. **Systemic predisposing conditions** include acute bacterial endocarditis, cyanotic heart disease (right-to-left shunt), and chronic pulmonary abscesses. **Local predisposing conditions** include mastoiditis, paranasal sinusitis, acute otitis, open fracture, and previous neurosurgery. CT/MRI scan characteristically shows a ring-enhancing lesion. Clinical manifestations include signs of increased intracranial pressure (headache, vomiting, and papilledema). Focal neurological deficits vary depending on the site of lesion.

Subacute sclerosing panencephalitis is a rare complication of measles (rubeola) virus infection. Persistent immune-resistant measles virus infection causes slow-virus encephalitis. The typical scenario is a child who had measles age <2 and then 6–15 years later develops progressive mental deterioration with seizures. Subacute sclerosing panencephalitis may be fatal in 1–2 years once it develops.

Creutzfeldt-Jakob disease (CJD) is the most common human transmissible spongiform encephalopathy due to a prion (a protein with the capacity to be an infectious agent) that can change the conformation of normal prion protein(s). This can lead to rapidly progressive dementia, memory loss, personality changes, and hallucinations.

- The **prion protein** (PrP) is a 30-kD protein normally present in neurons. It is encoded by a single-exon gene on chromosome 20. Its normal conformation is an α-helix: **PrP^{c}**. In disease states, PrP^{c} changes to a β-pleated sheet conformation: **PrP^{sc}**. A low rate of spontaneous change results in

sporadic cases of CJD. Mutations of PrP result in hereditary cases of CJD. PrP^{sc} facilitates conformational change of other PrP^{c} molecules into PrP^{sc}.

- PrP^{sc} is responsible for **cerebral pathologic changes**, characteristically resulting in spongiform change. This change is a fine vacuolization of the neuropil in the gray matter (especially cortex), which is due to large membrane-bound vacuoles within neuronal processes. There is an associated neuronal loss and astrogliosis. Kuru plaques are deposits of amyloid composed of altered PrP protein.
- About 85% of Creutzfeld-Jakob cases are sporadic, and 15% are familial. Affected patients are typically middle-aged to elderly patients who develop rapidly progressive dementia and memory loss with startle myoclonus or other involuntary movements. Typical EEG changes may be diagnostic. Death occurs within 6–12 months.
- Variant Creutzfeldt-Jacob disease occurs in younger patients and results from exposure to bovine spongiform encephalopathy.

Table 20-2. Prion Diseases

Disease	Infectious Agent	Host	Comments
Kuru	Prion	Human	Subacute spongiform encephalopathy (SSE); Fore Tribe in New Guinea; consuming infected brains
Creutzfeldt-Jakob	Prion	Human	SSE Genetic predisposition
Gerstmann-Straussler	Prion	Human	SSE
Fatal familial insomnia	Prion	Human	SSE
Scrapie	Prion	Sheep	SSE—scraping their wool off on fences

HIV-associated neurocognitive disorder (HAND) presents as cognitive decline with behavioral changes and motor symptoms. Diagnosis is based on clinical features and the exclusion of other etiologies.

CEREBROVASCULAR DISEASE

Bridge to Anatomy

The brain is highly dependent on a constant supply of oxygen and glucose from the cerebral arteries, which have collateral blood flow at the circle of Willis.

Cerebrovascular disease is the third most frequent cause of death in industrialized countries, and it is the leading cause of serious disability in the United States. Risk factors are similar to coronary artery disease.

- **Global cerebral ischemia** (diffuse ischemic encephalopathy) is caused by a fall in blood flow to the brain, due to processes such as shock, cardiac arrest, and hypotensive episodes. While the entire brain can be damaged, some regions have selective vulnerability, including Purkinje neurons, hippocampus, CA1 (Sommer sector), and pyramidal neurons of cortex. The pathology often includes infarcts in watershed areas, cortical laminar necrosis, and diffuse ischemic necrosis of neocortex. Global cerebral ischemia may lead to brain death.

- **Transient ischemic attack** (TIA) is due to small platelet thrombi or atheroemboli and is characteristically reversible, with symptoms lasting <24 hours.
- **Stroke** can be due to infarction (85% of all stroke cases) or hemorrhage (15% of all stroke cases).
- Infarction causes 85% of all stroke cases.
    - Can be due to **thrombotic occlusion** in the setting of atherosclerosis of the cerebral arteries; the thrombotic infarction is characteristically an anemic (white) infarct.
    - Can be due to **embolic occlusion**, most often due to thromboemboli from cardiac chambers and less frequently due to atheroemboli. Embolic infarction produces a hemorrhagic infarct. Small-vessel disease is a cause of small, lacunar infarcts or lacunae, and it is related to hypertension, resulting in hyaline arteriolosclerosis.
    - Atherosclerotic aneurysms are fusiform, involve the basilar artery, and present with infarction.
    - The pathology (i.e., morphological features of brain infarcts) of infarction is illustrated below. Clinical manifestations depend on the affected arterial distribution.

Clinical Correlate

Strokes frequently occur in the middle cerebral artery territory.

Table 20-3. Gross and Microscopic Changes Associated with Cerebral Infarction

Time	Gross Changes	Microscopic Changes
0–12 h	No changes	Minimal or no changes
12–24 h	Minimal changes	Red (hypereosinophilic) neurons with pyknotic nuclei
24–48 h	Indistinct gray-white matter junction	Neutrophilic infiltration
2–10 d	Friable tissue with marked edema	Histiocytic infiltration; neurons disappear
2–3 wk	Tissue liquefies	Liquefactive necrosis; histiocytes filled with products of myelin breakdown
3 wk–mo	Fluid-filled cavity demarcated by gliotic scar	Fluid-filled cavity; reactive astrocytes and lipid-laden macrophages
Years	Old cyst surrounded by gliotic scar	Astrogliosis surrounding a cyst

Note: Hemorrhagic infarct leads to erythrocyte degradation and hemosiderin deposition.

Hemorrhage causes 15% of strokes.

- **Intracerebral (intraparenchymal) hemorrhage** causes severe headache, frequent nausea/vomiting, steady progression of symptoms over 15–20 minutes, and coma. It is most frequently due to hypertension, and in those instances, it most commonly involves the basal ganglia, cerebellum, pons, and centrum semiovale.

Other causes include vascular malformations (especially arteriovenous malformations), cerebral amyloid angiopathy, neoplasms, vasculitides, abnormal hemostasis, hematological malignancies, infections, and diabetes mellitus.

- **Subarachnoid hemorrhage** is most frequently caused by ruptured berry aneurysm. Less frequent causes include extension of an intracerebral or subdural hematoma, vascular malformations, trauma, abnormal hemostasis, and tumors. Subarachnoid hemorrhage causes sudden headache ("worst headache of my life"), nuchal rigidity, neurological deficits on one side, and stupor.
  - **Berry aneurysms** are thin-walled saccular outpouchings, consisting of intima and adventitia only. They are the most frequent cause of subarachnoid hemorrhage. The most frequent sites are the anterior circle of Willis at branching points. Rupture is precipitated by a sudden increase in blood pressure; the prognosis after rupture is that one-third die, one-third recover, and one-third rebleed. The pathogenesis involves a congenital focal weakness of vessel media that is not identifiable at birth. Associated disorders include Marfan syndrome, Ehlers-Danlos type 4, and adult polycystic kidney disease. Hypertension and cigarette smoking predispose to formation.

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Figure 20-2. Large Berry Aneurysm Seen on Angiography of Circle of Willis

CNS TRAUMA AND HERNIATIONS

Cranial Cavity and Brain

Concussion is mild traumatic brain injury with a transient loss of brain function. The trauma is commonly due to a change in the momentum of the head (impact against a rigid surface). Concussion causes loss of consciousness and reflexes, temporary respiratory arrest, and amnesia for the event. The pathogenesis is uncertain. Parenchymal injuries may or may not be evident at autopsy.

Contusions are bruises of the brain tissue. Common sites of injury include crests of orbital gyri in frontal and temporal poles, in addition to *coup* (site of injury) and *contrecoup* (site diametrically opposite) injuries. Coup and contrecoup develop when the head is mobile at the time of impact.

- **Acute contusion** is characterized by hemorrhage of brain tissue in a wedge-shaped area.
- **Subacute contusion** shows necrosis and liquefaction of brain.
- **Remote contusion** causes a depressed area of cortex with yellow discoloration ("*plaque jaune*").

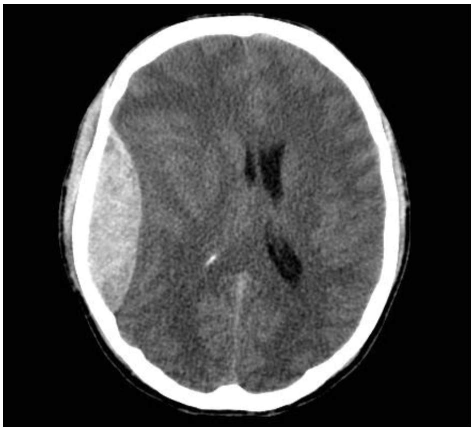

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Figure 20-3. Epidural Hematoma

Epidural hematoma (*See* Anatomy Lecture Notes.)

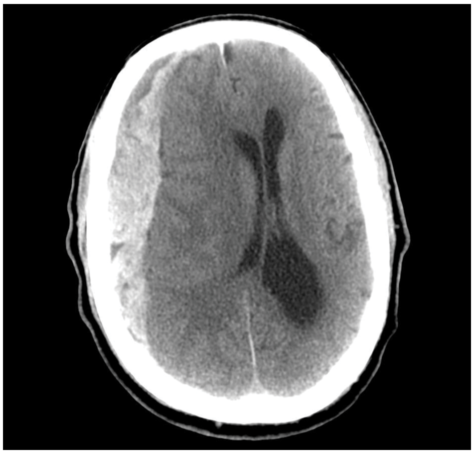

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Figure 20-4. Subdural Hematoma

Subdural hematoma is caused by the rupture of bridging veins (from the cerebral convexities to the sagittal sinus); it is usually traumatic in older individuals. Predisposing conditions include brain atrophy (due simply to aging) and abnormal

hemostatis. Symptoms include headache, drowsiness, focal neurological deficits, and sometimes dementia. It recurs frequently.

Diffuse axonal injury refers to damage to axons at nodes of Ranvier with impairment of axoplasmic flow. It causes coma after trauma without evidence of direct parenchymal injuries. There is a poor prognosis, related to duration of coma. The injury to the white matter is due to acceleration/deceleration forces with shearing of axons.

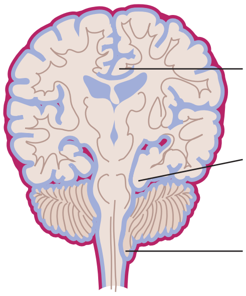
The histopathology shows axonal swellings appreciable in the white matter. It is diffuse, but with a predilection for the corpus callosum, periventricular white matter, and hippocampus, as well as cerebral and cerebellar peduncles.

Spinal cord injuries are usually traumatic, due to vertebral displacement. Symptomatology depends on the interruption of ascending and descending tracts.

- Lesions to thoracic segments or below cause paraplegia.
- Lesions to cervical segments cause tetraplegia.
- Lesions above C4 cause respiratory arrest due to paralysis of the diaphragm.

Chronic traumatic encephalopathy is a neurodegenerative disorder that occurs years or decades after a sports career with repetitive brain trauma. Neuropathological changes include neurofibrillary tangles, cerebellar atrophy and gliosis, hypopigmentation of the substantia nigra, and cavum septum pellucidum.

Cerebral Herniations



Cerebral Herniations

Subfalcine (cingulate gyrus) herniation occurs when the cingulate gyrus is displaced underneath the falx to the opposite side. Compression of the anterior cerebral artery can occur.

Transtentorial (uncal) herniation occurs when the uncus of the temporal lobe is displaced over the free edge of the tentorium. Clinical features include compression of the third nerve, ipsilateral pupillary dilatation, and infarction of the tissue supplied by the posterior cerebral artery. Advanced stages of transtentorial herniation can cause Duret hemorrhages within the central pons and midbrain.

Cerebellar tonsillar herniation occurs when there is displacement of cerebellar tonsils through the foramen magnum. Compression of the medulla may lead to cardiorespiratory arrest.

DEVELOPMENTAL ABNORMALITIES AND PERINATAL BRAIN INJURY

Neural tube defects are the most common developmental central nervous system abnormalities. They result from defective closure of the neural tube, and they tend to occur at the 2 extremities of the neuraxis. Folate deficiency is involved in the pathogenesis.

- **Anencephaly** is the absence of cranial vault. It is incompatible with life; babies die soon after birth.
- **Neural tube defects of the spinal cord** may take a variety of forms. Significant defects lead to paraplegia and urinary incontinence from birth.

- **Spina bifida occulta** is a bony defect of the vertebral arch.
- **Meningocele** is a bony defect with outpouching of meninges.
- **Meningomyelocele** is a defective formation of the bony arch with cystic outpouching of meninges, spinal cord, and spinal roots.
- **Myelocele** is a defective bony arch with complete exposure of the spinal cord.

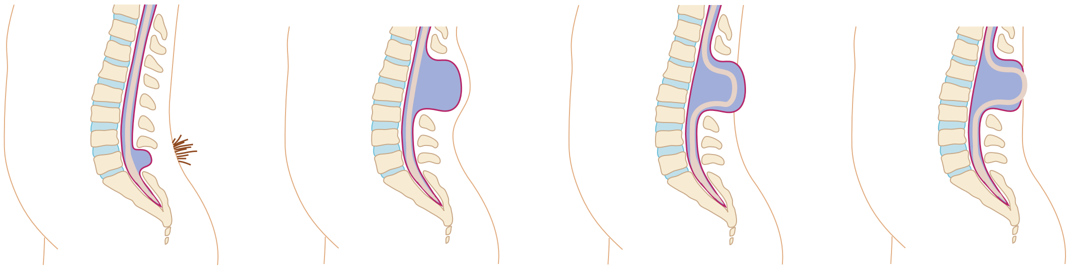
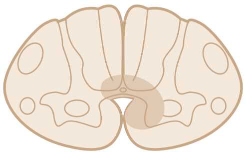


Figure 20-5. Neural Tube Defects of the Spinal Cord

Syringomyelia is an ependymal-lined, CSF-filled channel parallel to and connected with the central canal in the spinal cord. (*Hydromyelia* means the central canal is dilated with CSF.) About 90% of cases are associated with Arnold-Chiari type 2; the remaining 10% are posttraumatic or associated with intraspinal tumors. *Syrinx* (the cyst) enlarges progressively and destroys the spinal parenchyma. Symptoms include paralysis and loss of sensory functions. (*See* Anatomy Lecture Notes.)



Syringomyelia

Perinatal brain injury is injury to the brain during prenatal or immediately postnatal period. This is the most common cause of cerebral palsy, and it occurs most frequently in premature babies.

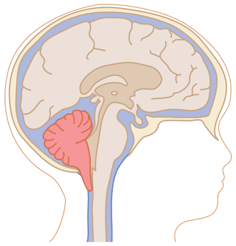
- **Germinal matrix hemorrhage** is hemorrhage localized in the germinal matrix due to its fragile vessels.
- **Periventricular leukomalacia** causes infarcts in watershed areas (periventricular white matter in the fetus).
- **Multicystic encephalopathy** refers to multiple brain infarcts occurring early in pregnancy.

Fetal alcohol syndrome is characterized by structural abnormalities (microcephaly, agenesis of the corpus callosum, cerebellar hypoplasia), functional impairments including learning disabilities, and neurological impairments including epilepsy.

Cerebellar Malformations

Cerebellar malformations have chromosomal, single-gene and complex inheritance. *FOXCI* deletions and duplications are associated with cerebellar vermis hypoplasia, mega-cisterna magna and Dandy-Walker malformation, the most common human cerebellar malformation.

- **Dandy-Walker malformation** is a non-communicating hydrocephalus with dilation of the fourth ventricle and hypoplasia of the cerebellar vermis.



Arnold-Chiari Malformation

- **Arnold-Chiari malformations**
  - **Type 1** (common) is a downward displacement of cerebellar tonsils and the medulla through the foramen magnum. This lesion is mostly asymptomatic.
  - **Type 2** is due to a faulty craniospinal junction, resulting in a small posterior fossa, with abnormal development of the cerebellar vermis and medulla leading to downward displacement. It is mostly symptomatic because of compression of the fourth ventricle with obstructive hydrocephalus.
  - Other frequently related manifestations include syringomyelia and lumbar meningomyelocele.

DEMYELINATING DISORDERS

Multiple sclerosis (MS) is a chronic relapsing-remitting disorder of probable autoimmune origin characterized by recurrent episodes of demyelination in the brain (including optic nerves) and spinal cord; it results in progressive neurological deficits.

- The overall prevalence of MS is 1/1,000, with higher prevalence in northern countries.
- Those who emigrate age >15 from areas of high prevalence to areas of low prevalence maintain their original risk.
- Women have 2x the risk of men.

Genetic and environmental factors contribute to the pathogenesis. HLA DR 15 confers genetic susceptibility. Environmental factors include viral infection, vitamin D deficiency, and smoking.

- **Acute lesions** on gross examination show well-circumscribed gray lesions (plaques), with bilateral distribution that is frequently periventricular. Histology shows chronic inflammation with phagocytosis of myelin by macrophages; axons are initially preserved.
- **Chronic lesions** have no inflammation, with axons showing remyelination. Remyelination is defective because myelin sheaths are thinner with shorter internodes.

During an acute attack, nerve conduction is entirely blocked, leading to acute neurological deficits. Chronic plaques are associated with slower nerve conduction, allowing for partial recovery. Recurrent attacks cause progressive neurological deterioration.

Clinical onset is typically in decades 3–4. About 85% of cases show a relapsing-remitting course; a minority of cases show primary progressive (slow deterioration) or progressive-relapsing (slow progression punctuated by acute exacerbations) course. Recovery from each episode of demyelination occurs in weeks or months.

Early symptoms include sensory problems, paresis, and visual dysfunction. As the disease progresses, other symptoms include fatigue, bladder dysfunction, spasticity and ataxia. Neuropsychological symptoms affect 40–60% of patients.

Diagnosis of MS requires the demonstration of the dissemination of disease in space and time. Clinical history, MRI, CSF studies and electrophysiological studies are important. Treatment is immunomodulatory drugs (e.g., interferon beta),

immunosuppressive therapies (e.g., mitoxantrone), and monoclonal antibodies (e.g., natalizumab). The latter is used as monotherapy in cases of relapsing MS; its use is linked to PML.

Central pontine myelinolysis (CPM) is a focal demyelination of the central area of the basis pontis. It probably derives from rapid correction of hyponatremia, and the condition is very often fatal. Patients at risk include the severely malnourished and alcoholics with liver disease.

DEGENERATIVE AND DEMENTING DISORDERS

Parkinson disease (PD) is a progressive neurodegenerative disease that involves genetic and environmental factors. The *SNCA* gene (alpha-synuclein) has been identified as a risk factor, and gene mutations and multiplications are associated with familial PD, but the majority of cases are sporadic. PD is due to loss of dopaminergic neurons in the substantia nigra, leading to tremor, rigidity, and akinesia.

- **Parkinson disease** is the idiopathic form.
- **Parkinson syndrome** is secondary to known injuries to the substantia nigra (e.g., infection, vascular condition, toxic insult).

Parkinson disease is common, affecting 2% of the population. Clinical onset is typically in decades 5–8. Loss of dopaminergic neurons is still unexplained, though theories emphasize oxidative stress. Pesticides and meperidine have been associated with increased risk, while smoking and caffeine are protective.

On gross examination there is pallor of the substantia nigra. Histology shows loss of pigmented (dopaminergic) neurons in the substantia nigra. Residual neurons show Lewy bodies, which are intracytoplasmic round eosinophilic inclusions that contain α-synuclein. Electron microscopy shows filaments most likely of cytoskeletal origin. There is also a secondary degeneration of dopaminergic axons in the striatum.

Loss of the extrapyramidal nigrostriatal pathway leads to inhibition of movement of proximal muscles and disruption of fine regulation of distal muscles. Involvement of the amygdala, cingulate gyrus and higher cortical regions causes dementia and psychosis.

About 60% of patients experience dementia 12 years after diagnosis; 50% also experience depression and psychosis. Those treated with medication (combination carbidopa and levodopa) and surgery (deep brain stimulation) will become refractory to therapy.

A clinical diagnosis is difficult to make early in disease because symptoms overlap with other conditions. Early symptoms include hyposmia, constipation, and fatigue. Key features are bradykinesia, rigidity, tremor and postural instability. Early in the disease course, a response to levodopa can help confirm the diagnosis. Imaging studies are not useful in most cases.

Huntington disease (HD) is an autosomal dominant disorder. It is characterized pathologically by the degeneration of GABAergic neurons of the caudate nucleus, and clinically by involuntary movements, cognitive decline, and behavioral changes.

- Affects those of northwestern European descent
- Has an incidence in high-prevalence regions of 1/12,000–20,000

- Gene (*HTT*), located on chromosome 4, codes for a protein called huntingtin
- Mutations due to expansion of unstable cytosine-adenine-guanine (CAG) repeats
- Shows features of anticipation and genomic imprinting

The pathophysiology is that loss of caudate nucleus GABAergic neurons removes inhibitory influences on extrapyramidal circuits, thus leading to chorea.

Clinical onset is typically in decades 3–5. The chorea is characterized by sudden, unexpected, and purposeless contractions of proximal muscles while awake. Psychiatric symptoms may predate motor symptoms. Disease progression leads to dependency and death.

Gross examination shows atrophy of the caudate nucleus with secondary ventricular dilatation. Histology shows loss of small neurons in the caudate nucleus followed by loss of the larger neurons.

A definitive diagnosis can be based on clinical symptoms with an affected parent. Otherwise, DNA determination is the gold standard. Prenatal diagnosis and preimplantation diagnostics are available. Treatment is medical therapeutics for chorea (dopamine receptor blocking or depleting agents).

Alzheimer disease (AD) causes 60% of all cases of dementia. It is the most common cause of dementia in people age >65.

- Incidence is 2% at age 65 and doubles every 5 years
- Risk factors include aging and significant head trauma
    - Aluminum is an epiphenomenon, not a risk factor
- Protective factors include high level of education and smoking

About 5–10% of AD cases are hereditary, early onset, and transmitted as an autosomal dominant trait. There are 3 genes that cause autosomal dominant AD:

- *APP* (amyloid precursor protein)
- Presenilin 1 and 2 (*PSEN1* and *2*)

Carriers of *APP* and *PSEN1* mutations develop early-onset AD. Other AD susceptibility genes have been identified. *APOE* is the largest effect locus for late-onset AD.

Table 20-4. Genetics of AD

Genes causing autosomal dominant AD	• *APP* • *PSEN1* • *PSEN2* All can cause early onset disease but *PSEN2* is associated with a broad range of onset ages.
Susceptibility genes	• Apolipoprotein E gene (*APOE*) – ε2 allele: associated with decreased risk – ε3 allele: neutral – ε4 allele: high-risk • *SORL1*

AD is characterized by amyloid-β deposition, neurofibrillary tangle formation, and neuronal degeneration.

- **Abnormal proteins**. Aβ amyloid is a 42-residue peptide derived from a normal transmembrane protein, the amyloid precursor protein (APP). There is also an abnormal tau (a microtubule-associated protein).
- **Neuritic plaques** have a core of Aβ amyloid and are surrounded by abnormal neurites.
- **Neurofibrillary tangles** are intraneuronal aggregates of insoluble cytoskeletal elements, mainly composed of abnormally phosphorylated tau forming paired helical filaments.
- **Cerebral amyloid angiopathy** is accumulation of Aβ amyloid within the media of small and medium-size intracortical and leptomeningeal arteries; it may occur by itself and cause intracerebral hemorrhage.
- Additional changes include granulovacuolar degeneration and Hirano bodies, which develop in the hippocampus and are less significant diagnostically.

Affected areas are involved in learning and memory. Lesions involve the neocortex, hippocampus, and several subcortical nuclei including forebrain cholinergic nuclei (i.e., basal nucleus of Meynert). The earliest and most severely affected are the hippocampus and temporal lobe. Small numbers of neuritic plaques and neurofibrillary tangles also form in intellectually normal aging persons.

Macroscopic changes include atrophy of affected regions, producing brains that are smaller (atrophic), with thinner gyri and wider sulci. Hippocampi and temporal lobes are markedly atrophic.

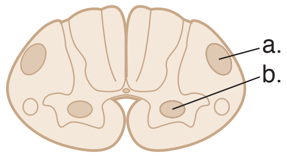
Clinical manifestations have insidious onset, typically beginning in decades 7–8. They include progressive memory impairment, especially related to recent events; alterations in mood and behavior; progressive disorientation; and aphasia (loss of language skills) and apraxia (loss of learned motor skills). Within 5–10 years patients become mute and bedridden.

No effective treatment is available for AD but there is mild improvement with inhibitors of acetylcholinesterase (e.g., tacrine).

Lewy body dementia is a progressive brain disease associated with the formation of Lewy bodies in neurons involving neocortex and subcortical nuclei. The etiopathogenesis is obscure, with no known risk factors; it is the second leading cause of degenerative dementia in the elderly.

The histopathological hallmark is the Lewy body. Neuron loss accompanies Lewy body formation. Sites involved include the neocortex (especially the limbic system and cingulate gyrus), and subcortical nuclei, including basal nucleus of Meynert, amygdala, and substantia nigra.

The involvement of the neocortex and substantia nigra is responsible for cognitive deterioration and parkinsonism. Clinical manifestations include memory loss, parkinsonism, and visual hallucinations. There is a possible treatment benefit from cholinesterase inhibitors.



ALS
a. Primary lateral sclerosis (corticospinal tract)
b. Progressive spinal muscular atrophy (ventral horn)

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset, progressive motor neuron disease.

The clinical diagnosis is supported by a biopsy of muscles. The etiopathogenesis is obscure; 5–10% of cases are hereditary, and a small number are caused by mutation of the gene encoding zinc-copper superoxide dismutase on chromosome 21.

- **Loss of upper motor neurons** produces hyperreflexia and spasticity. In some cases, involvement of cranial nerve nuclei also occurs.
- **Loss of lower motor neurons** produces weakness, atrophy, and fasciculations.

There is no cure for ALS. Ultimately, involvement of respiratory muscles will lead to death.

Friedreich ataxia is an autosomal recessive disorder which leads to degeneration of nerve tissue in the spinal cord, especially those sensory neurons connected to the cerebellum affecting muscle movement of the arms and legs. Mean onset is early childhood (age 10-15).

Friedreich ataxia is caused by the expansion of an unstable triplet nucleotide repeat (GAA repeats in the first intron) in the frataxin gene on chromosome 9. The frataxin protein is essential for mitochondrial function by helping in mitochondrial iron regulation; in the absence of frataxin, mitochondrial iron builds up, leading to free radical damage and mitochondrial dysfunction.

Clinical manifestations include gait ataxia, dysarthria, hand clumsiness, loss of sense of position, impaired vibratory sensation, and loss of tendon reflexes. There is an increased incidence of heart disorders and diabetes. Patients become wheelchair-bound by age 30–45.

Wilson disease (*See* Liver Pathology chapter.)

Acute intermittent porphyria is an autosomal dominant defect in porphyrin metabolism with deficient uroporphyrinogen synthase. Both porphobilinogen and aminolevulinic acid are increased. Urine is initially colorless but on exposure to light turns dark red. Patients may develop recurrent severe abdominal pain, psychosis, neuropathy, and dementia.

Vitamin B12 deficiency causes megaloblastic anemia, demyelination of the spinal cord posterior columns and lateral corticospinal tracts (subacute combined degeneration of the spinal tract). It also causes dementia and peripheral neuropathy.

Alcohol abuse causes generalized cortical and cerebellar atrophy, as well as Wernicke-Korsakoff syndrome. The neurologic disease is usually related to thiamine deficiency. There can be hemorrhages in the mamillary bodies and the walls of the third and fourth ventricles. Neuronal loss and gliosis may be prominent.

- **Wernicke encephalopathy** has reversible confusion, ataxia, and nystagmus.
- **Korsakoff psychosis** is more severe and has irreversible anterograde and retrograde amnesia.
- **Central pontine myelinolysis** may cause death.

CNS TUMORS

CNS tumors account for 20% of all pediatric tumors. Most pediatric tumors mainly arise in the posterior fossa, while adult tumors arise in the supratentorial region. Factors determining prognosis and response to therapy include the following:

- Age
- Tumor location
- Grade
- Extent of surgical resection
- Molecular subgroupings

The World Health Organization (WHO) grading system assigns grades I–IV, with grade IV tumors the most aggressive. While grade IV glioblastoma patients usually succumb to disease within a year, other patients with treatable grade IV tumors may survive 5 years. Metastasis of CNS tumors is rare.

Table 20-5. Primary Versus Metastatic (CNS) Tumors

Primary	Metastatic
Poorly circumscribed	Well circumscribed
Usually single	Often multiple
Location varies according to specific type	Location typically at the junction between gray and white matter

Tumors of Neuroepithelial Tissue

Tumors of neuroepithelial tissue are categorized as **astrocytic** tumors, **oligodendroglial** tumors, **ependymal** tumors, and **embryonal** tumors (further broken down as **medulloblastoma** or **CNS primitive neuroectodermal tumor**).

Astrocytoma originates from astrocytes and exhibits fibrillary background, immunoreactivity for glial fibrillary acidic protein (GFAP), and diffuse (ill-demarcated) pattern of growth.

- **Pilocytic astrocytoma** is a well-differentiated, benign astrocytic tumor that arises throughout the neuraxis; it is common in children and young adults. It is the most common benign CNS tumor in children.
- Sites of involvement include posterior fossa (cerebellum) and diencephalon
  - Radiographically, most show cystic lesion with a mural nodule
  - Histology shows spindly neoplastic astrocytes with long bipolar processes
  - Rosenthal fibers are thick, corkscrew-like eosinophilic structures that derive from hypertrophic processes of astrocytes
  - Posterior fossa tumors have favorable prognosis
  - Activating mutations in *BRAF* are common

- **Fibrillary (diffuse) astrocytoma** is a low-grade tumor that arises in the cerebral hemisphere of young to middle-aged adults and the brainstem of children.
    - IDH1 (immunostain) is positive.
- **Anaplastic astrocytoma** is cellular, pleomorphic and mitotically active.
- **Glioblastoma** is the most common CNS primary malignancy in adults.
    - Histology shows necrosis and/or vascular proliferation in addition to features seen in anaplastic astrocytoma.

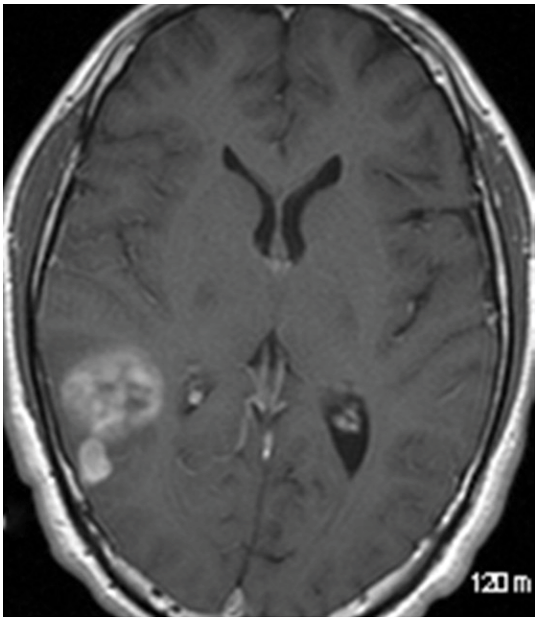


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Figure 20-6. White Mass in the Cerebral Cortex Showing a Glioblastoma Multiforme

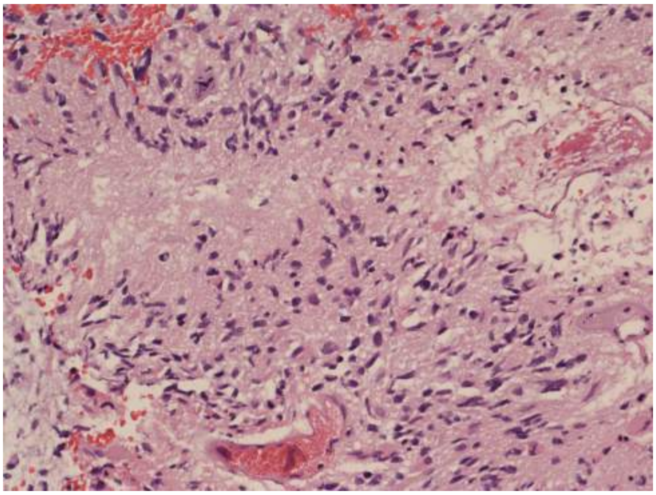

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Figure 20-7. Glioblastoma with Necrosis

The prognosis of astrocytomas varies.

- Well-differentiated astrocytomas grow slowly; affect younger patients
- Anaplastic astrocytomas and glioblastoma are aggressive; affect older patients; median survival for glioblastoma is 15 months

Oligodendroglioma occurs more often in adults than in children. Its cortical location may cause seizures. Histologically, perinuclear halos are a fixation artifact that is not seen on frozen section. This tumor is slow-growing. Tumors with deletions of 1p and 19q respond well to therapy.

Ependymoma is typically located in the fourth ventricle in children, where it presents with obstructive hydrocephalus. In adults the spinal cord is the most common site. Pseudorosettes are a helpful diagnostic feature on microscopic study. Multifocality in the spinal cord is associated with NF2. The prognosis depends on tumor location and adequacy of resection.

Embryonal (primitive) tumors are a group of small round cell tumors that occur predominantly in children. In the cerebellum, they are called medulloblastoma.

- Medulloblastoma is the most common malignant brain tumor in children.
- Molecular subgroupings are proving useful for prognosis; Wnt subgroup has the best prognosis.

Note

Glioblastoma multiforme has a tendency to cross the midline by involving the corpus callosum ("butterfly glioma").

Tumors of Cranial and Paraspinal Nerves

Schwannoma originates from Schwann cells of cranial or spinal nerves. The most frequent location is on CN 8 at the cerebellopontine angle (CPA). Schwannoma manifests characteristically with unilateral loss of hearing and tinnitus. The prognosis is good after surgical resection.

- Has spindly cells arranged in hypercellular Antoni A areas; alternating hypocellular Antoni B areas; and *Verocay bodies*, parallel rows of neoplastic Schwann cells.
- Neoplastic cells are immunoreactive for S-100.

Note

Bilateral acoustic schwannoma is pathognomonic of neurofibromatosis type 2.

Tumors of the Meninges

Meningioma is a tumor that originates from meningothelial cells of the arachnoid. It is common in adults (women > men) and rare in children. It is a dura-based mass that can recur if the brain has been invaded, but invasion is unusual. It has varied clinical features but commonly presents with headache, seizures, and neurological deficits.

- Histology shows cellular whorls and psammoma bodies. Many patterns are seen; the syncytial pattern is common.
- Abnormalities of chromosome 22 are sometimes present.
- Multiple meningiomas occur in NF2 patients.

The prognosis of meningioma is good, though tumors in some locations may not be amenable to complete resection.

Note the thin dark shadow around the lesion which occurs because the tumor is not actually invading the brain.

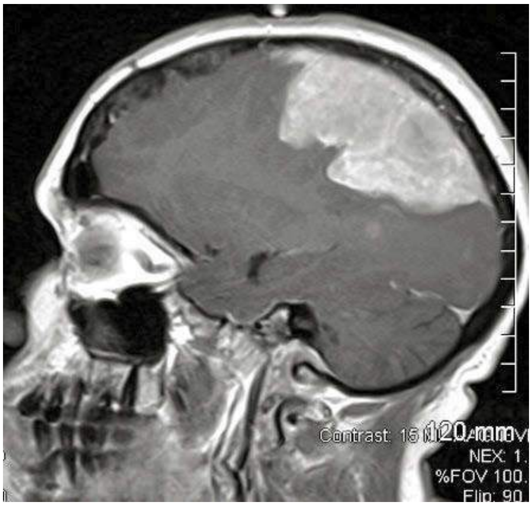

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Figure 20-8. Large Meningioma Pushing into the Cerebral Cortex.

Tumors of the Sellar Region

Craniopharyngioma arises from rests of odontogenic epithelium within the suprasellar/diencephalic region. It most commonly affects children and young adults. The most common presenting symptoms are headache, hypopituitarism, and visual field disturbances.

- Contains deposits of calcium, evident on x-ray
- Histology shows squamous cells and resembles **adamantinoma**, a bone tumor of unknown histological origin that is the most common tumor of the tooth
- Is benign but tends to recur after resection
- Beta-catenin (*CTNNB1*) gene mutations have been reported

Other Neoplasms

- **Lymphomas** are the most common CNS tumors in the immunosuppressed. Primary CNS lymphomas may be multiple, unlike other histologic types. They do not respond well to chemotherapy.
- **Germ cell tumors** are more common in children than adults. The germinoma is the most common histologic type. It resembles the seminoma of the testis and the dysgerminoma of the ovary; the cells are large with a prominent nucleolus. These tumors are radiosensitive.

Metastatic Tumors

About 25–50% of all CNS tumors are metastatic tumors from sources outside the CNS. **Carcinomas** are the most common.

Hematopoietic Pathology–White Blood Cell Disorders & Lymphoid and Myeloid Neoplasms

21

Learning Objectives

- ❑ Explain information related to reactive changes in white blood cells
- ❑ Describe lymphoid, mature B-cell, peripheral T-cell, and natural killer cell neoplasms
- ❑ Solve problems concerning Hodgkin lymphoma, acute leukemias, B and T lymphoblastic lymphoma/leukemia, and myeloid neoplasms
- ❑ Solve problems concerning diseases of histiocytes and dendritic cells
- ❑ Demonstrate understanding of mast cell diseases
- ❑ Answer questions about diseases of the spleen and thymus

REACTIVE CHANGES IN WHITE BLOOD CELLS

Leukocytosis

Leukocytosis is characterized by an elevated white blood cell count. It has the following features:

- **Increased neutrophils** (neutrophilia)
    - Increased bone marrow production is seen with acute inflammation associated with pyogenic bacterial infection or tissue necrosis
    - Increased release from bone marrow storage pool may be caused by corticosteroids, stress, or endotoxin
    - Increased bands ("left shift") noted in peripheral circulation
    - Reactive changes include Döhle bodies (aggregates of rough endoplasmic reticulum), toxic granulations (prominent granules), and cytoplasmic vacuoles of neutrophils
- **Increased eosinophils** (eosinophilia) occurs with allergies and asthma (type I hypersensitivity reaction), parasites, drugs (especially in hospitals), and certain skin diseases and cancers (adenocarcinomas, Hodgkin disease).
- **Increased monocytes** (monocytosis) occurs with certain chronic diseases such as some collagen vascular diseases and inflammatory bowel disease, and with certain infections, especially TB.
- **Increased lymphocytes** (lymphocytosis) occurs with acute (viral) diseases and chronic inflammatory processes.
    - **Infectious mononucleosis**, an acute, self-limited disease, which usually resolves in 4–6 weeks, is an example of a viral disease that causes lymphocytosis. The most common cause is Epstein-Barr virus (a herpesvirus)

though other viruses can cause it as well (heterophile-negative infectious mononucleosis is most likely due to cytomegalovirus).

- Age groups include adolescents and young adults ("kissing disease").
- The "classic triad" includes fever, sore throat with gray-white membrane on tonsils, and lymphadenitis involving the posterior auricular nodes. Another sign is hepatosplenomegaly.
- Complications include hepatic dysfunction, splenic rupture, and rash if treated with ampicillin.
- Diagnosis is often made based on symptoms. Lymphocytosis and a rising titer of EBV antibodies are suggestive of the infection. Atypical lymphocytes may be present in peripheral blood. Monospot test is often negative early in infection.

- **Increased basophils** are seen with chronic myeloproliferative disorders such as polycythemia vera.

Leukopenia

Leukopenia is characterized by a decreased white blood cell count. It has the following features:

- **Decreased neutrophils** can be due to decreased production (aplastic anemia, chemotherapy), increased destruction (infections, autoimmune disease such as systemic lupus erythematosus), and activation of neutrophil adhesion molecules on endothelium (as by endotoxins in septic shock).
- **Decreased eosinophils** are seen with increased cortisol, which causes sequestering of eosinophils in lymph nodes; examples include Cushing syndrome and exogenous corticosteroids.
- **Decreased lymphocytes** are seen with immunodeficiency syndromes such as HIV, DiGeorge syndrome (T-cell deficiency), and severe combined immunodeficiency (B- and T-cell deficiency); also seen secondary to immune destruction (systemic lupus erythematosus), corticosteroids, and radiation (lymphocytes are the most sensitive cells to radiation).

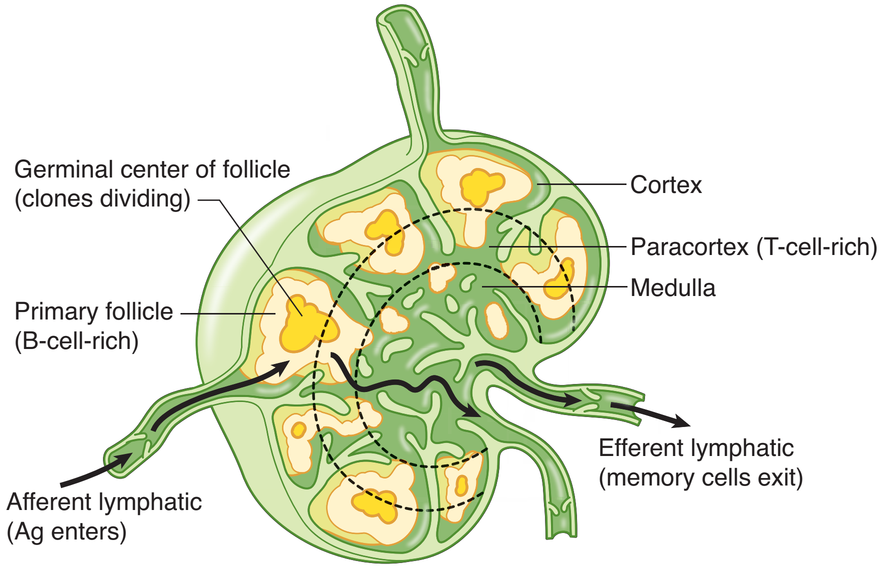

Figure 21-1. Lymph Node

Lymphadenopathy

Lymphadenopathy is lymph node enlargement due to reactive conditions or neoplasia.

- **Acute nonspecific lymphadenitis** produces tender enlargement of lymph nodes; focal involvement is seen with bacterial lymphadenitis. Microscopically, there may be neutrophils within the lymph node. Cat-scratch fever (due to *Bartonella henselae*) causes stellate microabscesses. Generalized involvement of lymph nodes is seen with viral infections.

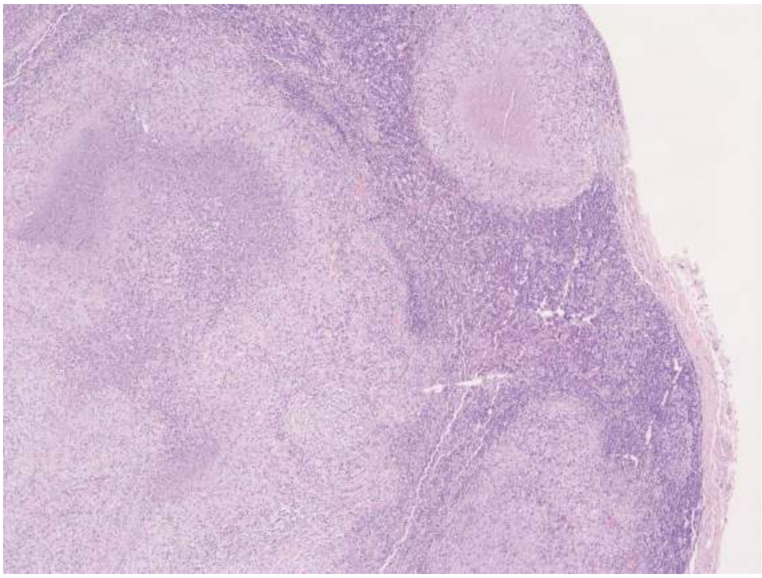

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Figure 21-2. Stellate Microabscesses in a Cat-Scratch Fever Lymph Node

- **Chronic nonspecific lymphadenitis** causes nontender enlargement of lymph nodes. Follicular hyperplasia involves B lymphocytes and may be seen with rheumatoid arthritis, toxoplasmosis, and early HIV infections. Paracortical lymphoid hyperplasia involves T cells and may be seen with viruses, drugs (Dilantin), and systemic lupus erythematosus. Sinus histiocytosis involves macrophages and, in most cases, is nonspecific; an example is lymph nodes draining cancers.
- **Neoplasia** usually causes nontender enlargement of lymph nodes. The most common tumor to involve lymph nodes is metastatic cancer (e.g., breast, lung, malignant melanoma, stomach and colon carcinoma), which is initially seen under the lymph node capsule. Other important causes of lymphadenopathy are malignant lymphoma and infiltration by leukemias.

LYMPHOID NEOPLASMS

Lymphoid neoplasia is grouped according to the 2008 WHO classification as follows (note that B and T lymphoblastic lymphoma/leukemia is grouped by the WHO with myeloid neoplasia):

- Mature B-cell neoplasms
- Mature T-cell and NK-cell neoplasms

- Hodgkin lymphoma
- Histiocytic and dendritic cell neoplasms
- Posttransplantation lymphoproliferative disorders

MATURE B-CELL NEOPLASMS

Chronic lymphocytic leukemia (CLL) and **small lymphocytic lymphoma (SLL)** are very similar; they both represent an abnormal proliferation of B cells. Patients who present with **lymph node findings** are classified as having SLL. Patients who present with **blood findings** are classified as having CLL; 50% of CLL patients also have lymph node involvement.

- CLL is the most indolent of all of the leukemias.
- Mean age at time of diagnosis is age 60.
- The malignant cells are nonfunctional, so patients develop hypogammaglobulinemia, leading to an increased risk of infections.
- CLL is associated with warm autoimmune hemolytic anemia (AIHA) (10% of cases), which will cause spherocytes to be observed in peripheral blood.
- CLL rarely transforms into a worse disease such as prolymphocytic leukemia or large cell lymphoma (Richter syndrome).

CLL and SLL can be categorized by the markers present on the B cells:

- **B-chronic lymphocytic leukemia cells** (95% of cases) have B-cell markers, such as CD19 and CD20. One T-cell marker, CD5, is also present. Also important is that the cells are CD23 positive and CD10 negative.
    - SLL occurs only as this type.
- **T-chronic lymphocytic leukemia cells** (5% of cases) have T-cell markers. The histology of affected lymph nodes reveals only a diffuse pattern (not nodular), but proliferation centers may also be present.
- Peripheral blood findings show increased numbers of normal-appearing lymphocytes. Numerous smudge cells (“parachute cells”) are also present; the smudge cells result from the fact that the neoplastic lymphocytes are unusually fragile.
- Bone marrow shows numerous normal-appearing neoplastic lymphocytes.

Hairy cell leukemia is a rare B-cell neoplasm that causes indolent disease in middle-aged Caucasian men. There can be a “dry tap” with bone marrow aspiration. Lymphocytes have “hairlike” cytoplasmic projections; the diagnostic stain is positive tartrate-resistant acid phosphatase (TRAP).

Physical examination shows a markedly enlarged spleen (splenomegaly) due to infiltration of red pulp by malignant cells.

Treatment is 2-chloro-deoxyadenosine (2-CdA), which inhibits adenosine deaminase (ADA) and increases levels of toxic deoxyadenosine.

Diffuse large B-cell lymphoma (DLBCL) (most common lymphoma worldwide) is a heterogeneous neoplasm with clinical and pathologic diversity. The most common type (DLBCL, NOS) occurs mainly in the elderly but can affect all ages; it can present with nodal or extranodal disease. DLBCL is an aggressive neoplasm but cure is possible in most cases with a combination of immunotherapy and chemotherapy.

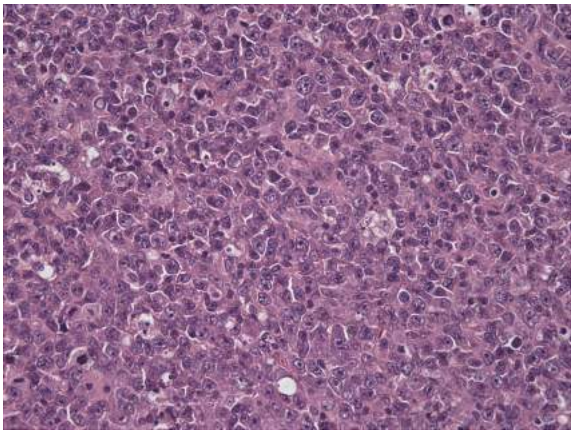

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Figure 21-3. Diffuse Large B-Cell Lymphoma

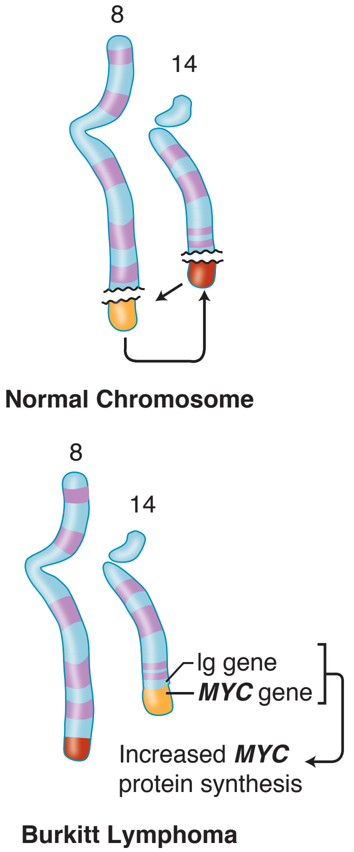
Follicular lymphoma (FL) (second most common lymphoma worldwide) is a neoplasm of germinal center-derived B-cells. Although subtypes exist, most cases have widespread disease with an indolent course; histologic transformation to a more aggressive non-Hodgkin lymphoma can occur.

Most FL cases have a characteristic translocation t(14;18) involving the immunoglobulin heavy chain gene and *BCL2* gene; some subtypes such as pediatric-type follicular lymphoma are t(14;18) negative.

Small noncleaved lymphoma (Burkitt lymphoma) is a high grade B-cell lymphoma. It is composed of intermediate-sized lymphoid cells with a "starry sky" appearance due to numerous reactive tingible-body macrophages (phagocytosis of apoptotic tumor cells). There is a characteristic t(8;14) translocation juxtaposing MYC to the immunoglobulin heavy chain locus in most cases.

- **African type:** endemic form
  - Involvement of mandible or maxilla is characteristic; is associated with Epstein-Barr virus
- **American type**: nonendemic, sporadic form
  - Involvement of the abdomen (such as bowel, retroperitoneum, or ovaries); has a high incidence in AIDS patients

Both endemic and sporadic forms of Burkitt lymphoma are seen most often in children and young adults.



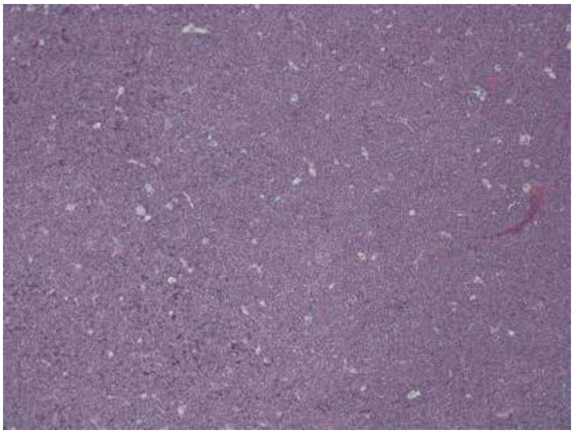


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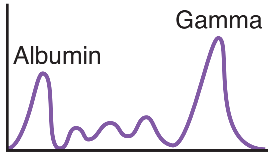
Figure 21-4. “Starry Sky” Appearance of Burkitt Lymphoma



Serum electrophoresis pattern of IgG myeloma with γ spike and reduced albumin

Mantle cell lymphoma (MCL) is a rare B-cell lymphoma in which the tumor cells arise from mantle zone B lymphocytes (positive for CD19, CD20, and CD5; negative for CD23). The characteristic translocation is t(11;14), involving *CCD1* and the heavy chain locus.

Marginal zone lymphoma (MALToma) is a diverse group of B-cell neoplasms that arise within lymph nodes, spleen, or extranodal tissue. It is associated with mucosa-associated lymphoid tissue (MALTomas). The lesion begins as a reactive polyclonal reaction and may be associated with previous autoimmune disorders or infectious disease (e.g., Sjögren disease, Hashimoto thyroiditis, *Helicobacter* gastritis). The lymphoma remains localized for long periods of time.

Multiple myeloma is a malignant neoplasm of plasma cells.

- Most common primary tumor arising in the bone marrow of adults
- Lab studies show increased serum protein with normal serum albumin; an M spike in serum electrophoresis is a monoclonal immunoglobulin spike—most commonly IgG (60%) and next most commonly IgA (20%)
- Bence Jones proteins are light chains that are small and can be filtered into urine.

Histologically, bone marrow shows increased plasma cells (>20% is characteristic). Peripheral blood may show rouleaux formation (“stack of coins”). Multiple lytic bone lesions are due to the osteoclastic activating factor. Lytic bone lesions cause hypercalcemia, bone pain, and increased risk of fracture.

Increased risk of infection is the most common cause of death. Other complications include renal disease (such as myeloma nephrosis) and primary amyloidosis (10% of patients) due to amyloid light (AL) chains. Increased amounts of IL-6 are associated with a poorer prognosis because survival of myeloma cells is dependent on IL-6.

Plasmacytoma is a solitary myeloma within bone or soft tissue.

- **Within bone**: precursor lesion that can later develop into myeloma
- **Outside bone** (extramedullary): usually found within upper respiratory tract

Monoclonal gammopathy of undetermined significance (MGUS) (an old name was benign monoclonal gammopathy). Serum M protein is found in 1–3% of

asymptomatic individuals age >50; the incidence increases with increasing age. The annual risk of developing a plasma cell dyscrasia, usually multiple myeloma, is 1–2% per year. MGUS may also evolve into Waldenström macroglobulinemia, primary amyloidosis, B-cell lymphoma, or CLL.

Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia) is a small lymphocytic lymphoma with plasmacytic differentiation. It is a cross between multiple myeloma and SLL.

Like myeloma, it has an M spike (IgM). Like SLL (and unlike myeloma), the neoplastic cells infiltrate many organs (e.g., lymph nodes, spleen, bone marrow). Also unlike multiple myeloma, there are no lytic bone lesions and there is no increase in serum calcium. Russell bodies (cytoplasmic immunoglobulin) and Dutcher bodies (intranuclear immunoglobulin) may be present.

- May have hyperviscosity syndrome, because IgM is a large pentamer
- Visual abnormalities may be due to vascular dilatations and hemorrhages in the retina
- Neurologic symptoms include headaches and confusion
- Bleeding and cryoglobulinemia can be due to abnormal globulins, which precipitate at low temperature and may cause Raynaud phenomenon

PERIPHERAL T-CELL AND NATURAL KILLER CELL NEOPLASMS

Peripheral T-cell lymphoma, unspecified is a “wastebasket” diagnostic category.

Adult T-cell leukemia/lymphoma (ATLL) is a malignant T-cell disorder (CD4-T cells) due to HTLV-1 infection. It is often seen in Japan and the Caribbean. Clinical symptoms include skin lesions, hypercalcemia, enlarged lymph nodes, hepatomegaly, and splenomegaly. Microscopically, characteristic hyperlobated “4-leaf clover” lymphocytes can be found in the peripheral blood.

Mycosis fungoides is a malignant T-cell disorder (CD4+ cells) that has a better prognosis than ATLL. It can present with a generalized pruritic erythematous rash (no hypercalcemia), which develops as a sequence of skin changes:

inflammatory eczematous stage → plaque stage → tumor nodule stage

Microscopically, atypical PAS-positive lymphocytes are present in the epidermis (epidermotropism); aggregates of these cells are called Pautrier microabscesses. If there is erythroderma and cerebriform Sézary cells are present in peripheral blood, the condition is called **Sézary syndrome**.

HODGKIN LYMPHOMA

Hodgkin lymphoma has some characteristics that are different from non-Hodgkin lymphoma.

- May present similar to infection (with fever)
- Spread is contiguous to adjacent node groups
- No leukemic state
- Extranodal spread is uncommon

The malignant cells are the diagnostic **Reed-Sternberg cells**; these malignant cells are intermixed with reactive inflammatory cells. The Reed-Sternberg cell is a large malignant tumor cell that has a bilobed nucleus with a prominent large inclusion-like nucleolus in each lobe.

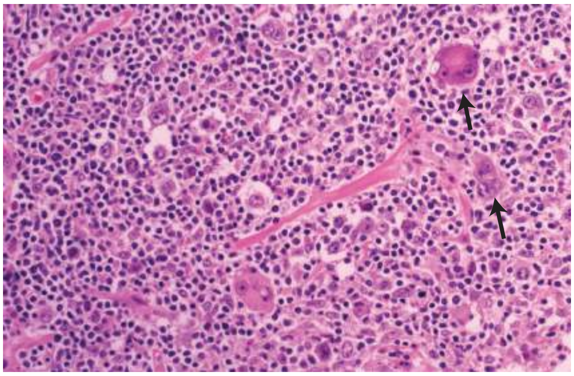


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Figure 21-5. Reed-Sternberg Cells (arrows) of Hodgkin Lymphoma Appear as Large Binucleate Cells with Macronucleoli

Hodgkin lymphoma classification:

- **Lymphocyte-rich** type (rare): composed primarily of reactive lymphocytes; associated with Epstein-Barr virus (40% of cases)
- **Lymphocyte-predominant** type: has lymphohistiocytic variants (L&H cells, called "popcorn cells") and a unique phenotype (CD45+, CD15-, CD30-, CD20+)
- **Mixed cellularity** type: occurs in middle-aged and older males; the increased number of eosinophils is related to IL-5 secretion
- **Lymphocyte-depleted** type: presents with abdominal adenopathy; Reed-Sternberg cells predominate
- **Nodular sclerosis** type (most common subtype (65–70% of cases)): only type in which females > males
    - Lymph node has broad collagen bands
    - Reed-Sternberg cell has clear space in the cytoplasm (lacunar cell)

Note

The classical Hodgkin lymphomas (nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte-depleted) share the immunophenotype CD45-, CD15+, CD30+.

Hodgkin lymphoma has a bimodal age group distribution (age late 20s and >50). Patients usually present with painless enlargement of lymph nodes. Poor prognosis is directly proportional to the number of Reed-Sternberg cells present. Survivors of chemotherapy and radiotherapy have increased risk for secondary non-Hodgkin lymphoma or acute leukemia.

Note

Hodgkin lymphoma patients who have symptoms (fever, night sweats, unexplained weight loss > 10%) have a worse prognosis.

ACUTE LEUKEMIAS

In acute leukemias, the peripheral blood has decreased mature forms and increased immature forms called **blasts**, which have immature chromatin with nucleoli. The bone marrow has increased immature cells (blasts). Acute symptoms are secondary to marrow failure, which can produce decreased erythrocytes (causing anemia and fatigue), decreased leukocytes (permitting infections and fever), and decreased platelets (inducing bleeding).

B AND T LYMPHOBLASTIC LYMPHOMA/LEUKEMIA

Acute Lymphoblastic Leukemia (ALL)

- **Karyotypic abnormalities:** Most pre-B-cell tumors are hyperdiploid. Translocations are common in both B-ALL and T-ALL.
- **Immunophenotyping.** Most tumors are positive for terminal deoxytransferase (TdT).
  - **B-cell lineage** classification is based on presence or absence of cytoplasmic or surface markers, including surface immunoglobulin (sIg) presence (mature B-ALL) and cytoplasmic μ presence (pre-BALL). The B-cell tumors almost always express B-cell molecules CD19 and CD10.
  - **T-cell lineage**: The majority of T-ALLs stain with CD2, CD3, CD5, and CD7.

B-ALL is more common in children; symptoms include fever, anemia, and bleeding. **T-ALL** is more common in adolescent males, who present with a mediastinal mass.

Lymphoblastic Lymphoma

Most cases of lymphoblastic lymphoma are T-cell neoplasms that are aggressive and rapidly progressive. Most patients are young men with a mediastinal mass (think thymus). The leukemic phase of lymphoblastic lymphoma is similar to T-ALL and some consider them the same entity. Most cells are CD1+, CD2+, CD5+, and CD7+.

MYELOID NEOPLASMS

Acute Myelogenous Leukemia

Acute myelogenous leukemia is a cancer of the myeloid line of blood cells. Median age at diagnosis is age 50. Symptoms include fatigue, unusual bleeding, and infections.

Lab findings: Myeloid blasts or promyelocytes represent at least 20% of the marrow cells. **Auer rods** (linear condensations of cytoplasmic granules) are characteristic of AML and are not found in normal myeloid precursors.

The WHO classification of AML (2008) is as follows:

- AML with recurrent genetic abnormalities (for some of these entities, the karyotype is diagnostic regardless of the blast percentage)
  - Promyelocytic leukemia has t(15;17)(q22;q12) with fusion gene PML/RARA and responds to all-transretinoic acid (ATRA); DIC is common
  - AML with either t(8;21)(q22;q22) or inv(16)(p13.1;q22)
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, not otherwise specified

Myelodysplastic Syndromes (MDS)

MDS are classified according to the number of blasts in the marrow. Dysplastic changes include Pelger-Huët cells ("aviator glasses" nuclei), ring sideroblasts, nuclear budding, and "pawn ball" megakaryocytes. MDS are considered preleukemias, so patients are at increased risk for developing acute leukemia.

Note

With some exceptions, lymphoblasts stain with PAS; myeloblasts are myeloperoxidase (MPO) positive.

Clinical Correlate

ALL is associated with infiltration of the CNS and testes (**sanctuary sites**). Thus, prophylactic radiation and/or chemotherapy to the CNS is used because malignant cells in the brain are protected from chemotherapy by the blood–brain barrier.

Clinical Correlate

Tumor lysis syndrome is a group of metabolic complications that can occur after treatment of neoplastic disorders. These complications, which include hyperkalemia, hyperphosphatemia, hyperuricemia, hypocalcemia, and acute renal failure, are caused by the breakdown products of dying cancer cells. Treatment is hydration and allopurinol.

MDS mainly affect older adults (age 50–70); they also predispose to infection, hemorrhage and anemia. Transformation to AML is common.

Myeloproliferative Neoplasms (MPN)

MPN are clonal neoplastic proliferations of multipotent myeloid stem cells. The bone marrow is usually markedly hypercellular (hence the name *myeloproliferative*). All cell lines are increased in number (erythroid, myeloid, and megakaryocytes).

- **Chronic myelogenous leukemia (CML)** is a clonal proliferation of pluripotent granulocytic precursor stem cells. In most cases it is associated with a *BCR-ABL* fusion gene due to a balanced (9;22) translocation; however, this Philadelphia chromosome is not specific to CML.

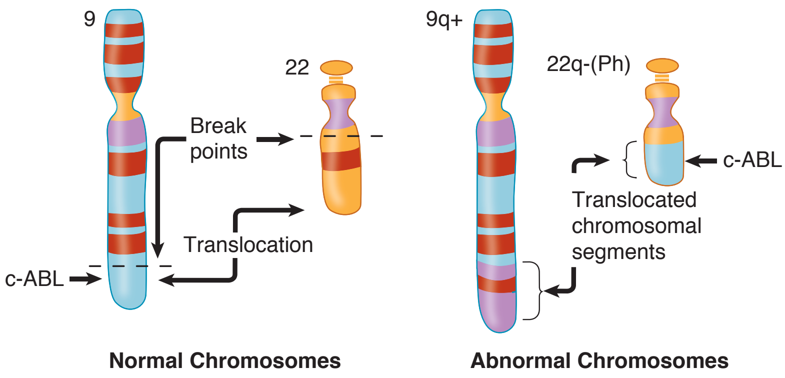

Figure 21-6. Translocation of Chromosomal Segments between Chromosomes 9 and 22

  - CML has an insidious onset (i.e., chronic) and causes massive splenomegaly. Progression is typically slow (50% develop accelerated phase <5 years), unless blast crisis develops (very poor prognosis; doesn't respond to chemotherapy). In blast crisis, 70% of cases show myeloid blasts and 30% show lymphoid blasts.
  - Microscopically, the bone marrow is hypercellular, with all cell lines increased in number. Peripheral leukocytosis is present, including markedly increased neutrophils (and bands and metamyelocytes), as well as increased eosinophils and basophils (as in the other MPS).
  - Treatment is imatinib mesylate, which blocks the P210 tyrosine kinase protein produced by the translocation. Hematopoietic stem cell transplantation is also used.
- **Polycythemia vera** is a stem cell disorder with trilineage (erythroid, granulocytic, megakaryocytic) proliferation. It may develop into a "spent phase" with myelofibrosis. It causes an increased risk for acute leukemia. Phlebotomy is therapeutic.

  Polycythemia vera characteristically shows the following:

  - Increased erythroid precursors with increased red cell mass
  - Increased hematocrit
  - Increased blood viscosity, which can cause deep vein thrombosis and infarcts

  - Decreased erythropoietin, but erythrocytes have increased sensitivity to erythropoietin and overproliferate
  - Increased basophils. Histamine release from basophils can cause intense pruritus and gastric ulcer (bleeding may cause iron deficiency).
  - Increased eosinophils (like all of the MPS)
  - High cell turnover can cause hyperuricemia, resulting in gout. Other clinical characteristics include plethora (redness) and cyanosis (blue).
- **Essential thrombocythemia** is characterized by increased megakaryocytes (and other cell lines) in bone marrow. Peripheral blood smear shows increased platelets, some with abnormal shapes. There are also increased leukocytes. Clinical signs include excessive bleeding and occlusion of small vessels.
- **Myelofibrosis (MF) with myeloid metaplasia** has unknown etiology (agnogenic).
  - Marrow fibrosis is secondary to factors released from megakaryocytes, such as platelet-derived growth factor (PDGF).
  - Bone marrow aspiration may be a "dry tap." The biopsy specimen shows hypocellular marrow with fibrosis (increased reticulin). The fibroblasts are a polyclonal proliferation and are not neoplastic.
  - There is an enlarged spleen due to extramedullary hematopoiesis (myeloid metaplasia). Peripheral smear shows leukoerythroblastosis (immature white cells and nucleated red cells) with teardrop RBCs. High cell turnover causes hyperuricemia and gout.

Note

The most common site for extramedullary hematopoiesis is the spleen.

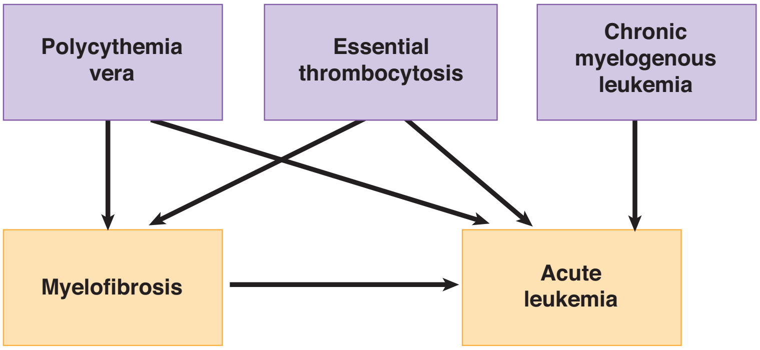

Figure 21-7. Natural History of the Myeloproliferative Syndromes

DISEASES OF HISTIOCYTES AND DENDRITIC CELLS

Langerhans histiocytosis is common in children. It can affect many sites, including skin, bone, CNS (diabetes insipidus), and lungs. Biopsy is required for diagnosis. The Langerhans cells are CD1a positive and on electron microscopy show cytoplasmic Birbeck granules (tennis racket–shaped organelles).

- Multisystem variant: Letterer-Siwe disease (marrow involvement can be fatal)
- Hand-Schuller-Christian triad is calvarial involvement, diabetes insipidus, and exophthalmos
- Unisystem variant: eosinophilic granuloma (most often found in bone)

MAST CELL DISEASES

Mastocytosis occurs from birth to adulthood; adult-onset cases are more severe. The WHO separates mastocytosis into 3 categories:

- **Cutaneous mastocytosis**, seen in children, regresses over time. Mast cell degranulation enzymes (histamine and tryptase) cause pruritus.
- **Systemic mastocytosis** is a clonal proliferation of mast cells associated with a KIT mutation; ≥1 organs are involved, as are the skin and bone marrow. The WHO now classifies systemic mastocytosis as one of the myeloproliferative neoplasms.
    - Variants include some rare leukemias.
    - Diagnosis is made with bone marrow biopsy and molecular analysis.
- **Localized extracutaneous mast cell neoplasms** can be benign (mastocytoma) or malignant (sarcoma).

DISEASES OF THE SPLEEN AND THYMUS

Splenomegaly (splenic enlargement) can be caused by multiple things:

- Vascular congestion (portal hypertension)
- Reactive hyperplasia of white pulp (autoimmune disorder, infectious mononucleosis, malaria)
- Infiltrative disease (metastatic non-Hodgkin lymphoma, primary amyloidosis, leukemia)
- Accumulated macrophages in red pulp (Gaucher, Niemann-Pick disease)
- Extravascular hemolysis
- Extramedullary hematopoiesis in splenic sinusoids

Hypersplenism will result in thrombocytopenia.

Splenic dysfunction will result in a loss of ability to remove damaged red cells, which leads to Howell-Jolly bodies in peripheral red blood cells. Splenectomized, asplenic, and hyposplenic individuals are at risk for infection (sepsis, peritonitis), particularly due to *Streptococcus*, *Haemophilus*, and *Salmonella*.

Thymomas are low-grade tumors of the thymic epithelium with many histologic patterns. Recent large case series have shown that tumor behavior does not always correlate with histopathological features.

True thymic hyperplasia is enlargement of a histologically normal thymus; it can occur as a complication of chemotherapy.

Thymic lymphoid hyperplasia shows germinal center hyperplasia.

Female Genital Pathology 22

Learning Objectives

- ❑ Demonstrate understanding of the pathology of the vulva, vagina, cervix, uterus, and ovary
- ❑ Solve problems concerning the placenta

VULVA

Non-Neoplastic Disorders

- **Lichen sclerosis** is caused by epidermal thinning and dermal changes which cause pale skin in postmenopausal women. There is a small risk of progression to squamous cell carcinoma (SCC).
- In **lichen simplex chronicus**, a chronic scratch/itch cycle produces the white plaques seen clinically. These plaques are characterized microscopically by squamous cell hyperplasia and dermal inflammation.

Infections

- **Human papillomavirus (HPV)** causes warty lesions (condylomata acuminata) and precursor dysplastic lesions of squamous cell carcinoma called vulvar intraepithelial neoplasia (VIN). Vulvar HPV is commonly subtype 6 and 11 and therefore has low oncogenic potential.
- **Herpes simplex virus (HSV)**. Most cases of vulvar herpes are caused by HSV-2. Painless vesicles progress to pustules and painful ulcers.
- **Syphilis** is a sexually transmitted disease caused by *Treponema pallidum*. The primary lesion is a chancre, a painless ulcer that does not scar after healing.
- **Molluscum contagiosum** is a viral disease caused by a DNA poxvirus. It presents as smooth papules and has characteristic cytoplasmic viral inclusions.
- **Bartholin gland abscess** is a polymicrobial infection requiring drainage or excision.

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Figure 22-1. Severe Case of Condyloma Acuminata

Tumors

- **Papillary hidradenoma** is a benign tumor of modified apocrine sweat glands of the labia majora or interlabial folds. It occurs along the milk line and may ulcerate, mimicking carcinoma. Papillary hidradenoma is histologically similar to an intraductal papilloma of the breast.
- **Extramammary Paget disease of the vulva** usually involves the labia majora, and it causes an erythematous, crusted rash that is characterized microscopically by intraepidermal malignant cells with pagetoid spread. This form of Paget disease is not usually associated with underlying tumor.
- **Squamous cell carcinoma** is the most common malignancy of the vulva. The most common form occurs in women age >60. The less common form occurs in younger women with HPV serotypes 16 and 18.
- **Melanoma** can occur on the vulva, and must be differentiated from lentigo simplex which is more common.

VAGINA

- **Vaginal adenosis and clear cell adenocarcinoma** are rare conditions with increased risk in females exposed to diethylstilbestrol (DES) *in utero*.
- **Embryonal rhabdomyosarcoma** (sarcoma botryoides) affects infants and young children (age <5), in whom it can cause a polypoid, "grapelike," soft tissue mass that protrudes from the vagina. Microscopically, the mass is characterized by polypoid epithelial growth with an underlying immature (cambium) proliferation of spindle-shaped tumor cells with rare cross-striations. Tumor cells are positive for desmin.
- **Primary forms of vaginal squamous cell carcinoma** are usually related to HPV infection; secondary forms are more common and are usually due to extension from a cervical cancer. Treatment is radiotherapy.
- **Rhabdomyoma** is a benign skeletal muscle tumor that can involve the vagina. It occurs in middle-aged women.

Clinical Correlate

DES was used in high-risk pregnancies from 1940–1970, after which time vaginal adenosis and clear cell carcinoma were discovered in the female offspring. Vaginal adenosis is a benign condition that is presumed to be a precursor of clear cell carcinoma.

- **Gartner duct cyst** is a cyst of the lateral wall of the vagina that is due to persistence of a mesonephric (Wolffian) duct remnant. Urinary tract abnormalities may exist.
- **Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome** is congenital absence of the upper part of the vagina and uterus. Patients present with primary amenorrhea.
- **Vaginitis/vaginosis:** All of the following conditions require vaginal swab for definitive diagnosis; molecular diagnostic tests may be indicated in certain situations.
  - **Vulvovaginal candidiasis** can occur spontaneously or from antibiotic therapy; it is not usually sexually transmitted. Symptoms include discharge and pruritis. Yeast cells and pseudohyphae are seen on microscopy. Antimycotics are therapeutic.
  - **Bacterial vaginosis (BV)** is implicated in preterm labor and pelvic inflammatory disease. Some patients are asymptomatic. BV is a sexually transmitted bacterial infection of polymicrobial origin (although it used to be attributed only to *Gardnerella vaginalis*); recurrence rate is high after treatment with antibiotics. “Clue cells” are squamous cells coated with coccobacilli that may be seen microscopically in swab material.
  - ***Trichomonas vaginalis*** is a sexually transmitted motile protozoan. Most infected people are asymptomatic. It can also cause cervicitis, but “strawberry cervix” is not a consistent diagnostic feature. Antibiotics are therapeutic.

CERVIX

Pelvic inflammatory disease (PID) is an ascending infection (sexually transmitted disease) from the cervix to the endometrium, fallopian tubes, and pelvic cavity. The infecting organisms are most frequently nongonococcal organisms, including *Chlamydia*, *Mycoplasma hominis* and endogenous flora. Broad-spectrum antibiotics are therapeutic.

The distribution of disease includes the endometrium (endometritis), fallopian tubes (salpingitis), and pelvic cavity (peritonitis and pelvic abscesses). **Fitz-Hugh–Curtis syndrome** (perihepatitis) can occur, characterized by “violin-string” adhesions between the fallopian tube and liver capsule. Symptoms include the following:

- Vaginal discharge (cervicitis)
- Vaginal bleeding and midline abdominal pain (endometritis)
- Bilateral lower abdominal and pelvic pain (salpingitis)
- Abdominal tenderness and peritoneal signs (peritonitis)
- Pleuritic right upper quadrant pain (perihepatitis)

Complications of PID include tubo-ovarian abscess; tubal scarring (increasing risk of infertility and ectopic tubal pregnancies), and intestinal obstruction secondary to fibrous adhesions.

Clinical Correlate

Tubal ectopic pregnancies usually occur in the ampulla of the fallopian tube. Tubal rupture will cause severe, acute lower abdominal pain.

Table 22-1. Malignant Tumors of the Lower Female Genital Tract in the U.S.

Order of Incidence	Order of Greatest Mortality
1. Endometrial cancer	1. Ovarian cancer
2. Ovarian cancer	2. Endometrial cancer
3. Cervical cancer	3. Cervical cancer

Cervical carcinoma is most commonly squamous cell carcinoma but can also be adenocarcinoma or small cell neuroendocrine carcinoma. It is the third most common malignant tumor of the lower female genital tract in the United States, with peak incidence at ages 35–44. Risk factors include the following:

- Early age of first intercourse
- Multiple sexual partners
- Multiple pregnancies
- Oral contraceptive use
- Smoking
- STDs (including human papilloma virus)
- Immunosuppression

Human papilloma virus infection is the most important risk factor, with high-risk types being 16, 18, 31, and 33, and having viral oncoproteins E6 (binds to p53) and E7 (binds to Rb).

The precursor lesion is **cervical intraepithelial neoplasia (CIN)**, which is increasing in incidence and occurs commonly at the squamocolumnar junction (transformation zone). Cervical intraepithelial lesions show a progression of changes on histologic examination:

- Low grade SIL (squamous intraepithelial lesion)
- High grade SIL
- Carcinoma in situ
- Superficially invasive squamous cell carcinoma
- Invasive squamous cell carcinoma

Squamous cell carcinoma of the cervix may be asymptomatic or may present with postcoital vaginal bleeding, dyspareunia, and/or malodorous discharge. To establish the diagnosis, the Papanicolaou (**Pap**) test is useful for early detection, and colposcopy with biopsy for microscopic evaluation.

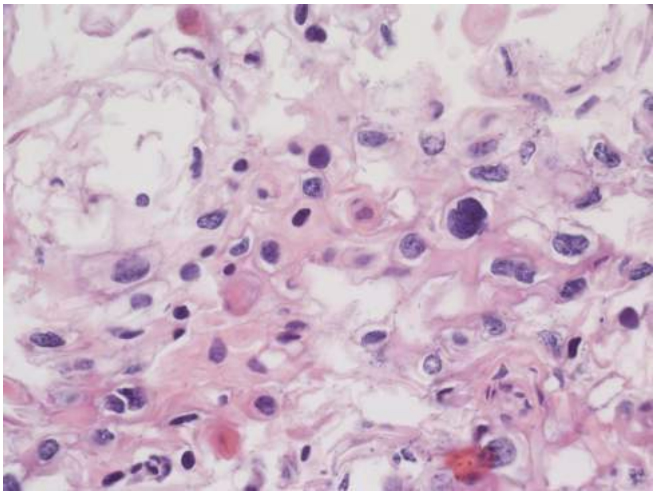

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Figure 22-2. Cervix Biopsy Showing Squamous Cells with Nuclear Hyperchromasia and Perinuclear Vacuoles, Characteristic of HPV Cytopathic Effect

Acute cervicitis and chronic cervicitis are common and usually nonspecific inflammatory conditions.

- Acute cervicitis is often caused by *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *Candida*, and herpes simplex type 2.
- A specific, severe form of chronic cervicitis (**follicular cervicitis**) can be caused by *C. trachomatis*; it can result in neonatal conjunctivitis and pneumonia in infants delivered vaginally through an infected cervix.

Cervical polyp is a common non-neoplastic polyp that can be covered with columnar or stratified squamous epithelium.

UTERUS

Endometritis is inflammation of the endometrial lining in the uterus. It can be acute or chronic.

- **Acute endometritis** is an ascending infection from the cervix; it is associated with pregnancy and abortion.
- **Chronic endometritis** is associated with PID and intrauterine devices; plasma cells are seen in the endometrium.

Endometriosis is the presence of endometrial glands and stroma outside the uterus. It most commonly affects women of reproductive age. Common sites of involvement are the ovaries, ovarian and uterine ligaments, pouch of Douglas, serosa of bowel and urinary bladder, and peritoneal cavity. It can present with chronic pelvic pain, dysmenorrhea and dyspareunia, rectal pain and constipation, abnormal uterine bleeding, or infertility.

Grossly, endometriosis causes red-brown serosal nodules (an *endometrioma* is an ovarian "chocolate" (hemolyzed blood) cyst).

Leiomyoma (fibroid), the most common tumor of the female genital tract, is a benign, smooth muscle tumor of the myometrium. Leiomyomas have a high incidence in African Americans, though they are common across all populations. Their growth is estrogen-dependent.

Leiomyomas may present with menorrhagia, abdominal mass, pelvic/back pain, suprapubic discomfort, or infertility and spontaneous abortion.

Grossly, leiomyomas form well-circumscribed, rubbery, white-tan masses with a whorled, trabeculated appearance on cut section. Leiomyomas are commonly multiple, and may have subserosal, intramural, and submucosal location. The malignant variant is leiomyosarcoma.

Endometrial hyperplasia refers to a histological proliferation of endometrial glands with 2 important histopathologic categories:

- **Benign endometrial hyperplasia** shows uniform remodeling of glands with cyst formation.
- **Endometrial intraepithelial neoplasia** shows crowded architecture and cytologic alteration on biopsy.
    - Patients are at high risk for endometrial adenocarcinoma.
    - Treatment options include total hysterectomy or progestin therapy with biopsy surveillance.

Endometrial adenocarcinoma is the most common malignant tumor of the lower female genital tract. It most commonly affects postmenopausal women who present with abnormal uterine bleeding. Risk factors are mostly related to estrogen:

- Early menarche and late menopause
- Nulliparity
- Hypertension and diabetes
- Obesity
- Chronic anovulation
- Estrogen-producing ovarian tumors (granulosa cell tumors)
- ERT and tamoxifen
- Endometrial hyperplasia (complex atypical hyperplasia)
- Lynch syndrome (colorectal, endometrial, and ovarian cancers)

Endometrial adenocarcinoma typically forms a tan polypoid endometrial mass; invasion of myometrium is prognostically important.

- Endometrioid adenocarcinoma (most common histological type): associated with *PTEN* mutations
- Serous tumors: associated with *TP53* mutations

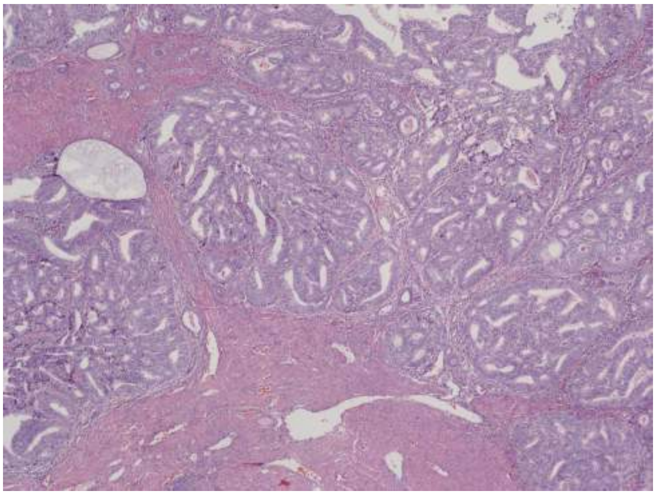

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Figure 22-3. Endometrial Carcinoma Invading the Myometrium

Less common types of uterine malignancy include **leiomyosarcoma**, a malignant, smooth muscle tumor, and **carcinosarcoma**, which contains both malignant stromal cells and endometrial adenocarcinoma.

Adenomyosis is an invagination of the deeper layers of the endometrium into the myometrium, which causes menorrhagia and dysmenorrhea.

Anovulation can cause abnormal uterine bleeding, especially in women near menarche and menopause. Biopsy shows glandular and stromal breakdown in a background of proliferative phase endometrium.

OVARY

Polycystic ovarian disease (Stein-Leventhal syndrome) is an endocrine disorder of unknown etiology showing signs of androgen excess (clinical or biochemical), oligoovulation and/or anovulation, and polycystic ovaries.

Accurate diagnosis requires exclusion of other endocrine disorders that might affect reproduction. Patients are usually young women of reproductive age who present with oligomenorrhea or secondary amenorrhea, hirsutism, infertility, or obesity. Treatment is lifestyle change and hormone therapy.

Lab studies show elevated luteinizing hormone (LH), low follicle-stimulating hormone (FSH), and elevated testosterone. Gross examination is notable for bilaterally enlarged ovaries with multiple cysts; microscopic examination shows multiple cystic follicles.

Note

- The Pap test has reduced the incidence of cervical cancer in the United States.
- There is no screening test for ovarian cancer.

Epithelial Ovarian Tumors

Epithelial ovarian tumors are the most common form of ovarian tumor. Risk factors include nulliparity, family history, and germline mutations.

Previously, epithelial tumors were characterized by histology into the categories cystadenoma (benign), borderline, and cystadenocarcinoma. Now, **serous tumors** are classified as **low grade** and **high grade** for prognostic significance.

- Low-grade serous tumors are associated with *KRAS*, *BRAF*, or *ERB2* mutations.
- Most high-grade serous tumors have *TP53* mutations.

The most common malignant ovarian tumor is **serous cystadenocarcinoma**. Hereditary risk factors include *BRCA1* (breast and ovarian cancers) and Lynch syndrome.

- Well-differentiated serous tumors show psammoma bodies and a lining similar to that of the fallopian tube.

Mucinous tumors commonly have goblet cells like intestinal mucosal cells.

CA 125 can be used to follow treatment.

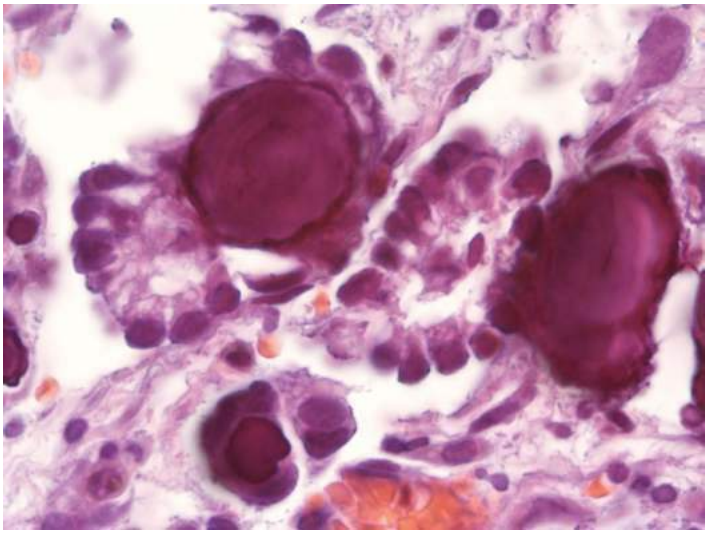

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Figure 22-4. Psammoma Bodies (Concentric Calcifications) in a Serous Tumor

Ovarian Germ Cell Tumors

- **Teratoma** (dermoid cyst)
    - Vast majority (>95%) of ovarian (but not testicular) teratomas are benign; commonly occurs in early reproductive years
    - Include elements from all 3 germ cell layers: ectoderm (skin, hair, adnexa, neural tissue), mesoderm (bone, cartilage), and endoderm (thyroid, bronchial tissue)
    - Complications include torsion, rupture, and malignant transformation
    - Can contain hair, teeth, and sebaceous material

- The term *struma ovarii* is used when there is a preponderance of thyroid tissue
- Immature teratoma is characterized by histologically immature tissue

- **Dysgerminoma**
  - Malignant; commonly occurs in children and young adults
  - Risk factors include Turner syndrome and disorders of sexual development
  - Gross and microscopic features are similar to seminomas
  - Are radiosensitive; prognosis is good

Ovarian Sex Cord–Stromal Tumors

- **Ovarian fibroma**
  - Most common stromal tumor; forms a firm, white mass
  - **Meigs syndrome** refers to the combination of fibroma, ascites, and pleural effusion.
- **Granulosa cell tumor**
  - Potentially malignant, **estrogen-producing** tumor
  - Presentation depends on age:
    - Prepuberal patients with juvenile granulosa cell tumor present with precocious puberty
    - Reproductive age patients present with irregular menses
    - Postmenopausal patients present with vaginal bleeding
  - Complications include endometrial hyperplasia and cancer
  - Tumor forms a yellow-white mass that microscopically shows polygonal tumor cells and formation of follicle-like structures (Call-Exner bodies)
- **Sertoli-Leydig cell tumor** (androblastoma) is an **androgen-producing tumor** that presents with virilization, usually in young women.

Primary sites for **metastatic tumor** to the ovary include breast cancer, colon cancer, endometrial cancer, and gastric "signet-ring cell" cancer (Krukenberg tumor).

Table 22-2. Origins of Common Ovarian Neoplasms

	Surface Epithelial Cells	Germ Cell	Sex Cord–Stroma	Metastasis to Ovaries
Age group affected	20+ years	0–25+ years	All ages	Variable
Types	• Serous tumor • Mucinous tumor • Endometrioid tumor • Clear cell tumor • Brenner tumor	• Teratoma • Dysgerminoma • Endodermal sinus tumor • Choriocarcinoma	• Fibroma • Granulosa–theca cell tumor • Sertoli-Leydig cell tumor	
Overall frequency	65–70%	15–20%	5–10%	5%
Percentage of malignant ovarian tumors	90%	3–5%	2–3%	5%

PLACENTA

Hydatidiform mole (molar pregnancy) is a tumor of placental trophoblastic tissue. Incidence in the United States is 1 per 1,000 pregnancies, with an even higher incidence in Asia. Women ages <15 and >40 are at increased risk.

- **Complete mole** results from fertilization of an ovum that *lost* all of its chromosomal material, so that all chromosomal material is derived from sperm.
  - 90% of the time, the molar karyotype is 46,XX
  - 10% of the time, the molar karyotype includes a Y chromosome
  - The embryo does not develop
- **Partial mole** results from fertilization of an ovum (that has not lost its chromosomal material) by 2 sperms, one 23,X and one 23,Y.
  - Results in a triploid cell 69, XXY (23,X [maternal] + 23,X [one sperm] + 23,Y [the other sperm])
  - The embryo may develop for a few weeks

Patients with hydatidiform mole typically present with the following:

- Excessive uterine enlargement ("size greater than dates")
- Vaginal bleeding
- Passage of edematous, grape-like soft tissue
- Elevated beta-human chorionic gonadotropin (β-hCG)

Microscopically, molar tissue will show edematous chorionic villi, trophoblast proliferation, and fetal tissue (only in partial mole). Diagnosis is by U/S. Treatment is endometrial curettage and following of β-hCG levels.

Table 22-3. Partial Mole Versus a Complete Mole

	Partial Mole	Complete Mole
Ploidy	Triploid	Diploid
Number of chromosomes	69	46 (All paternal)
β-hCG	Elevated (+)	Elevated (+++)
Chorionic villi	Some are hydropic	All are hydropic
Trophoblast proliferation	Focal	Marked
Fetal tissue	Present	Absent
Invasive mole	2%	10%
Choriocarcinoma	Rare	2%

Invasive mole is a mole that invades the myometrium of the uterine wall.

Choriocarcinoma is a malignant germ cell tumor derived from the trophoblast that forms a necrotic and hemorrhagic mass. Almost 50% arise from complete moles. The most common presentation is a rising or plateaued titer of hCG after a molar pregnancy, abortion, or ectopic pregnancy.

Microscopically, choriocarcinoma shows proliferation of cytotrophoblasts, intermediate trophoblasts, and syncytiotrophoblasts. Hematogenous spread can occur, with seeding of tumor to lungs, brain, liver, etc. Treatment is chemotherapy.

Placental site trophoblastic tumor is a tumor of intermediate trophoblast which usually presents <2 years after pregnancy with bleeding and an enlarged uterus. Treatment is surgical; it does not respond well to chemotherapy.

In **ectopic pregnancy**, the fetus implants outside the normal location, most often in the fallopian tube, and less often in the ovaries or abdominal cavity. The fetus almost never survives. The mother is at risk for potentially fatal intra-abdominal hemorrhage. Risk factors include scarring of fallopian tubes from PID, endometriosis, and decreased tubal motility.

Enlarged placenta is common with maternal diabetes mellitus, Rh hemolytic disease, and congenital syphilis.

Succenturiate lobes are accessory lobes of the placenta which may cause hemorrhage if torn away from the main part of the placenta during delivery.

Placental abruption is partial premature separation of the placenta away from the endometrium, with resulting hemorrhage and clot formation. Risk factors include hypertension, cigarette use, cocaine, and older maternal age.

Placenta previa describes when the placenta overlies the cervical os. Vaginal delivery can cause the placenta to tear, with potentially fatal maternal or fetal hemorrhage.

In **placenta accreta**, the placenta implants directly in the myometrium rather than in endometrium. Hysterectomy is required after delivery to remove the rest of the placenta.

Twin placentation

- **Fraternal twins** always have 2 amnions and 2 chorions; placental discs are usually separate, but can grow together to appear to be a single placental disc.
- **Identical twins** have a variable pattern in the number of membranes and discs due to variations in the specific point in embryonic development at which the twins separated. Twin-twin transfusion syndrome can occur if (a) there is only one placental disc and (b) one twin's placental vessels connect to the other twin's placental vessels.
- **Conjoined twins** are always identical twins with one amnion, one chorion, and one disc, though there are rare reports of diamniotic placentation.

Preeclampsia is a condition of new onset hypertension and either proteinuria or end-organ dysfunction after 20 weeks gestation in a previously normotensive woman. It is linked to abnormal uteroplacental blood flow.

- The term **eclampsia** is used when the patient has seizures not attributable to other causes.
- **HELLP syndrome** is a rare complication of preeclampsia characterized by hemolysis, elevated liver enzymes, and low platelets.

Breast Pathology 23

Learning Objectives

- ❑ Explain information related to mastitis
- ❑ Demonstrate understanding of fibrocystic changes
- ❑ Solve problems concerning benign and malignant neoplasms
- ❑ Answer questions about gynecomastia

MASTITIS

Mastitis is an infection of the breast tissue.

Acute mastitis causes an area of erythema and firmness in the breast, commonly during lactation. The most common infecting organism is *S. aureus*. The breast is often biopsied to differentiate the condition from inflammatory carcinoma, another painful breast condition. Microscopically there is acute and chronic inflammation with abscess formation in some cases.

Fat necrosis is often related to trauma or prior surgery, and it may produce a palpable mass or a discrete lesion with calcifications on mammography. Microscopic changes include fat necrosis, chronic inflammation, hemosiderin deposits and fibrosis with calcification.

FIBROCYSTIC CHANGES

Fibrocystic changes (formerly called *fibrocystic disease*) are a group of very common, benign changes that can be classified as **proliferative** (having an increase in the glandular elements or epithelial cells) or **nonproliferative**. Because they carry varying degrees of risk for breast cancer, it is important to identify each type histologically.

Fibrocystic changes primarily affect women in their reproductive years. The changes most often involve the upper outer quadrant and may produce a palpable mass or nodularity.

- **Fibrosis** may mimic a tumor on clinical exam and U/S.
- **Cysts** can usually be diagnosed by U/S.
- **Apocrine metaplasia** is often seen in cyst walls.
- **Microcalcifications** occur in benign and malignant processes.
- **Ductal hyperplasia** is classified as usual or atypical on the basis of cytology and microlumen architecture; **atypical ductal hyperplasia** is differentiated from DCIS on the basis of microscopic extent.

- **Atypical lobular hyperplasia** is differentiated from LCIS histologically on the basis of the percentage of acini involved.
- **Sclerosing adenosis** is distinguished from carcinoma histologically by the preservation of the myoepithelial layer.

Table 23-1. Nonproliferative Versus Proliferative Fibrocystic Changes

Nonproliferative	Proliferative Changes
Fibrosis Cysts (blue-domed) Apocrine metaplasia Microcalcifications	Ductal hyperplasia ± atypia Sclerosing adenosis Atypical lobular hyperplasia

Table 23-2. Relative Risk of Developing Breast Cancer with Fibrocystic Change

Relative Risk	Fibrocystic Change
No increase	Fibrosis, cysts, apocrine metaplasia, adenosis
1.5–2×	Sclerosing ductal hyperplasia, papillomas
4–5×	Atypical ductal or lobular hyperplasia

Table 23-3. Features That Distinguish Fibrocystic Change from Breast Cancer

Fibrocystic Change	Breast Cancer
Often bilateral	Often unilateral
May have multiple nodules	Usually single
Menstrual variation	No menstrual variation
May regress during pregnancy	Does not regress during pregnancy

BENIGN NEOPLASMS

Fibroadenoma is the most common benign breast tumor in women age <35. It causes a palpable, round, movable, rubbery mass, which on cross-section shows small, cleft-like spaces. Microscopically, the mass shows proliferation of benign stroma, ducts, and lobules.

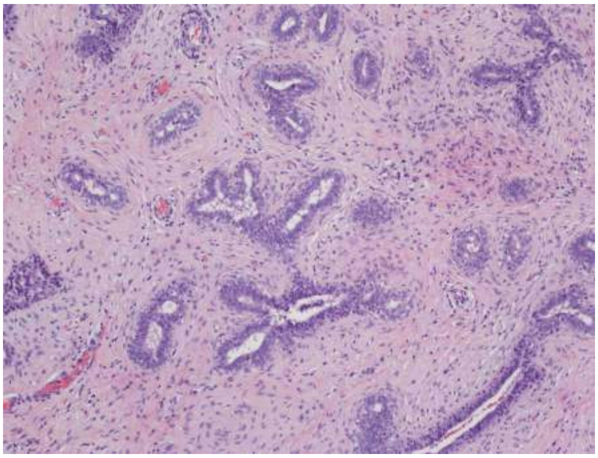

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Figure 23-1. Fibroadenoma

Phyllodes tumor (cystosarcoma phyllodes) usually involves an older patient population (age 50s) and can be benign or malignant. Local recurrence is common but the incidence of metastasis is low. Microscopically, the mass shows increased stromal cellularity, clefts lined by epithelium, stromal overgrowth, and irregular margins.

Intraductal papilloma commonly presents as a bloody nipple discharge. Microscopically, papilloma causes benign papillary growth within lactiferous ducts or sinuses; the myoepithelial layer is preserved.

MALIGNANT NEOPLASMS

Carcinoma of the breast is the most common cancer in women and affects 1 in 9 women in the United States. It is also the second most common cause of cancer death. The incidence is increasing and is higher in the United States than in Japan. Many risk factors have been identified.

The incidence increases with the following factors:

- Age
- Unusually long/intense exposure to estrogens (long length of reproductive life, nulliparity, obesity, exogenous estrogens)
- Presence of proliferative fibrocystic changes, especially **atypical hyperplasia**
- First-degree relative with breast cancer

Hereditary influences are thought to be involved in 5–10% of breast cancers, with important genes as follows:

- ***BRCA1*** (error-free repair of DNA double-strand breaks) chromosome 17q21
- ***BRCA2*** (error-free repair of DNA double-strand breaks) chromosome 13q12.3
- ***TP53*** germline mutation (Li-Fraumeni syndrome)

Carcinoma in situ and risk of invasive carcinoma. About 35% of women with untreated DCIS will develop invasive cancer, usually in the same quadrant of the breast. About 35% of women with LCIS will develop invasive lobular or ductal carcinoma, in either breast.

Breast cancer is most common in the upper outer quadrant. Gross examination of a breast cancer typically shows a stellate, white-tan, gritty mass. Clinically, it can cause:

- Mammographic calcifications or architectural distortion
- Palpable solitary painless mass
- Nipple retraction or skin dimpling
- Fixation of breast tissue to the chest wall

Paget disease of the nipple is an intra-epidermal spread of tumor cells from an underlying ductal carcinoma in situ or invasive ductal carcinoma. The tumor cells often lie in lacunae, and there can be a dermal lymphocytic infiltrate.

Histologic variants of breast cancer are as follows:

- **Preinvasive lesions** include ductal carcinoma in situ (**DCIS**) and lobular carcinoma in situ (**LCIS**). Preservation of the myoepithelial cell layer distinguishes them from their invasive counterparts.
- **Invasive (infiltrating) ductal carcinoma** is the most common form (>80% of cases). Microscopically, it shows tumor cells forming ducts within a desmoplastic stroma. About 70% of cases are ER/PR positive and 30% overexpress HER2.

Note

As per current practice guidelines, all primary invasive breast cancers are evaluated for ER/PR status and HER2 expression.

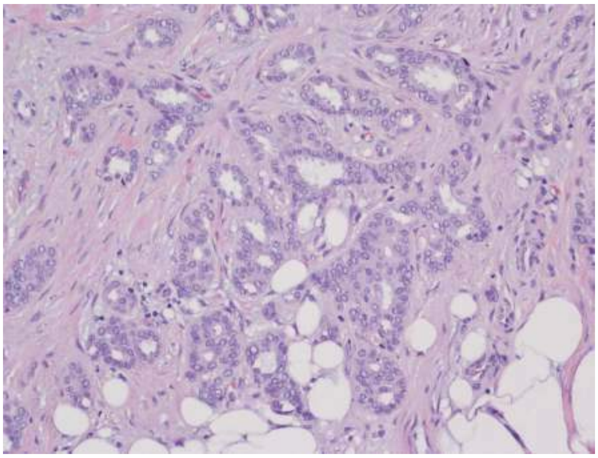

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Figure 23-2. Invasive Ductal Carcinoma

- **Invasive (infiltrating) lobular carcinoma** (5–10% of cases) is characterized by small, bland tumor cells forming a single-file pattern.
- Multifocal and bilateral disease occurs commonly.
  - About 50% are ER/PR-positive; these tumors do not overexpress HER2.
- **Mucinous (colloid) carcinoma** is characterized microscopically by clusters of bland tumor cells floating within pools of mucin. It has a better prognosis.
  - Hormone receptors are positive; these tumors do not overexpress HER2.
- **Tubular carcinoma** rarely metastasizes and has an excellent prognosis.
  - Hormone receptors are positive; these tumors do not overexpress HER2.
- **Medullary carcinoma** is characterized microscopically by pleomorphic tumor cells forming syncytial groups surrounded by a dense lymphocytic host response. It has a better prognosis.

- Hormone receptors are negative; the tumors do not overexpress HER2.

- **Inflammatory carcinoma** is related to tumor invasion into the dermal lymphatics with resulting lymphatic edema; it presents with red, warm, edematous skin. The prognosis is poor.
  - The term ***peau d'orange*** is used when the thickened skin resembles an orange peel. This is caused by the accentuation of the attachments of the suspensory ligaments of Cooper to the dermis.

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Figure 23-3. Advanced Breast Cancer with Peau d'Orange Skin Involvement

Mammary Paget disease (Paget disease of the nipple) is commonly associated with an underlying invasive or in situ ductal carcinoma. It may present with ulceration, oozing, crusting, and fissuring of the nipple and areola. Microscopic examination shows intraepidermal spread of tumor cells (Paget cells), with the cells occurring singly or in groups within the epidermis; there is often a clear halo surrounding the nucleus.

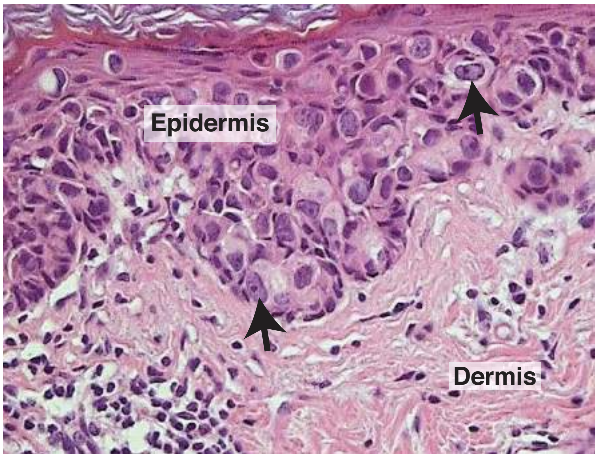

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Figure 23-4. Paget Disease of the Nipple

Note the intraepidermal tumor cells (arrows) with prominent nucleoli and dermal lymphocytic infiltrate.

The prognosis of breast cancer depends on the following:

- Axillary lymph node status as determined by sentinel node biopsy (SNB) or axillary dissection. In most cases, SNB is recommended to evaluate clinically tumor-free regional nodes.
- Size of tumor
- Histological type and grade of tumor
- ER/PR receptor status is used to select patients for endocrine forms of therapy.
- Overexpression of HER2/neu is associated with more aggressive behavior than other types of breast cancer; patients may respond to therapy with trastuzumab.

Treatment of breast cancer depends on the stage and other tests.

- Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) as measured by ELISA are used to guide treatment decisions with node-negative breast cancer, along with multiparameter gene expression analysis.
- Cancer antigen 15-3 (CA 15-3), cancer antigen 27.29 (CA 27.29), and carcinoembryonic antigen (CEA) are used to monitor patients with metastatic disease undergoing therapy.

Although the majority of cancers in the breast are primaries, **cancer from other organs can spread to the breast**. Lung cancer may spread by contiguity or via the lymphatics.

GYNECOMASTIA

Gynecomastia is a unilateral or bilateral benign breast enlargement in a male. It is usually caused by an altered androgen-estrogen balance that favors estrogen effect. Microscopically, it is characterized by:

- Ductal epithelial hyperplasia
- Ductal elongation and branching
- Proliferation of periductal fibroblasts
- Increase in vascularity in the involved tissue

Clinical Correlate

Causes of gynecomastia can be:

- Physiologic (newborn infants, pubescent adolescents, and elderly)
- Pathologic (cirrhosis, Klinefelter syndrome, pituitary tumors such as prolactinomas)
- Side effect of drugs (spironolactone and ketoconazole)

Male Pathology 24

Learning Objectives

- ❑ Explain information related to the pathology of the penis and testes
- ❑ Solve problems concerning testicular cancer
- ❑ Solve problems concerning prostate disease

PENIS

Malformations of the penis include epispadias and hypospadias. Both may be associated with undescended testes. **Epispadias** is a urethral opening on the dorsal surface of the penis, while **hypospadias** is a urethral opening on the ventral surface. Both have an increased risk of urinary tract infection and infertility.

Balanitis/balanoposthitis is inflammation of the glans penis, and the glans and foreskin, respectively. Causes include poor hygiene and lack of circumcision.

Peyronie disease is penile fibromatosis resulting in curvature of the penis during erection.

Condyloma acuminatum is a warty, cauliflower-like growth, with the causative agents most frequently being HPV serotypes 6 and 11.

Squamous cell carcinoma (SCC) is uncommon in the United States, and is often related to infection with HPV serotypes 16 and 18. There is an increased risk in uncircumcised males (multicentric carcinoma in situ). Precursor lesions include Bowen disease, bowenoid papulosis, and erythroplasia of Queyrat (a red plaque with carcinoma in situ histology).

Priapism is a persistent painful erection that can be caused by sickle cell anemia (causes blood sludging in penis), trauma, and drugs (e.g., trazodone).

Erectile dysfunction (ED). Causes of impotence include psychological factors, decreased testosterone, vascular insufficiency (most common cause age >50), neurologic disease (multiple sclerosis, diabetic neuropathy, radical prostatectomy), some medications (leuprolide, methyldopa, finasteride, psychotropic medications), hypothyroidism, prolactinoma, and penile disorders.

TESTES

Varicocele is a dilated pampiniform venous plexus and internal spermatic vein, usually on the left side. It may cause infertility. Clinically, it resembles a “bag of worms” superior to the testicle.

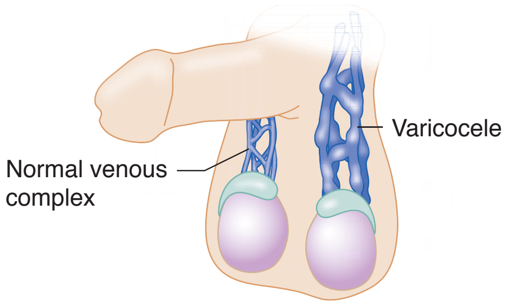

Figure 24-1. Varicocele

Hydrocele refers to fluid within the tunica vaginalis.

Spermatocele is an epididymal cyst containing sperm. On physical examination it transilluminates.

Epididymitis presents with fever and gradual onset of scrotal pain.

- Acute epididymitis that affects men age <35 is often caused by *N. gonorrhoeae* or *C. trachomatis*.
- Acute epididymitis that affects men age >35 is often caused by *E. coli* or *Pseudomonas*.
- Chronic epididymitis can be caused by TB.

Orchitis presents with sudden onset of testicular pain and fever. It is frequently viral, particularly due to the mumps virus.

Testicular torsion is twisting of the spermatic cord; may be associated with physical activity or trauma; and is a clinical emergency that can cause painful hemorrhagic infarction leading to gangrene.

Cryptorchidism is a failure of one or both testes to descend; the undescended testes are most commonly found in the inguinal canal. The undescended testes have an increased risk for developing seminoma.

Male infertility

- Decreased sperm count due to **primary testicular dysfunction** can be caused by Leydig cell dysfunction or seminiferous tubule dysfunction.
- Decreased sperm count due to **secondary hypogonadism** can be caused by pituitary and hypothalamic dysfunction.
- Inability of sperm to exit the body in sufficient numbers may be caused by **obstruction of the vas deferens** or **disordered ejaculation**.

TESTICULAR CANCER

Testicular cancer typically presents with a firm, painless testicular mass; nonseminomatous tumors may present with widespread metastasis. Caucasians have a higher incidence than African Americans.

Clinical Correlate

The most common regional metastatic lymph nodes for testicular cancer are the **para-aortic lymph nodes**, with lymphatic drainage following the gonadal vessels to the retroperitoneum.

Risk factors include:

- Cryptorchidism (3–5 times increased risk)
- Testicular dysgenesis (testicular feminization and Klinefelter syndrome)
- Positive family history

Clinically, U/S typically shows a hypoechoic intratesticular mass. Serum tumor marker studies can be helpful in confirming the diagnosis. Treatment is radical orchiectomy and possible chemotherapy/radiotherapy. Staging includes examination of the surgically resected specimen, including a lymph node dissection, along with imaging studies and lab tests.

Serum markers are used to monitor disease.

- AFP is produced by yolk sac tumors.
- β-hCG is produced by choriocarcinoma and any tumor with syncytiotrophoblastic giant cells.
- LDH is used to measure tumor burden.

Germ Cell Tumors

Germ cell tumors are usually hyperdiploid.

Seminoma is the most common germ cell tumor in adults, with mean age 40. It is characteristically sensitive to both chemotherapy and radiation, and has an excellent prognosis (early stage seminoma has 95% cure rate). A variant is spermatocytic seminoma, a disease of older men, also with an excellent prognosis.

On gross examination the tumor has a pale tan, bulging cut surface. Microscopic exam shows sheets of monotonous cells (with clear cytoplasm and round nuclei) separated by fibrous septae. Lymphocytes, granulomas, and giant cells may be seen.

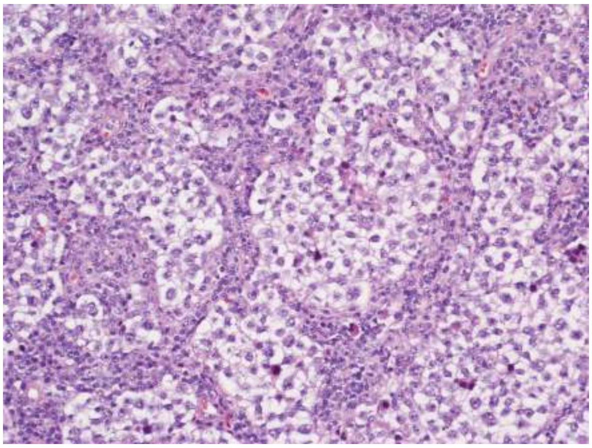

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Figure 24-2. Seminoma

Embryonal carcinoma is more aggressive than seminoma and affects adults ages 20–40. It causes bulky masses with hemorrhage and necrosis. Microscopy shows large primitive cells.

Choriocarcinoma is a highly malignant tumor that often has widespread metastasis at the time of diagnosis; hematogenous spread to lungs and liver is particularly common. The often small tumor has extensive hemorrhage and necrosis. Microscopically, syncytiotrophoblasts and cytotrophoblasts are seen.

Yolk sac tumor (endodermal sinus tumor) is the most common germ cell tumor in children; in pediatric cases, the prognosis is good. In adults, the prognosis may depend on the other histologic types that are admixed. Microscopically, yolk sac tumors show numerous patterns. Schiller-Duval bodies are glomeruloid structures.

Teratoma often causes cystic masses which may contain cartilage and bone. Microscopically, mature teratoma usually contains ectodermal, endodermal, and mesodermal tissue in a haphazard arrangement. Immature elements contain embryonic tissue. Prepubertal cases are benign regardless of immature elements; teratomas in adults have malignant potential.

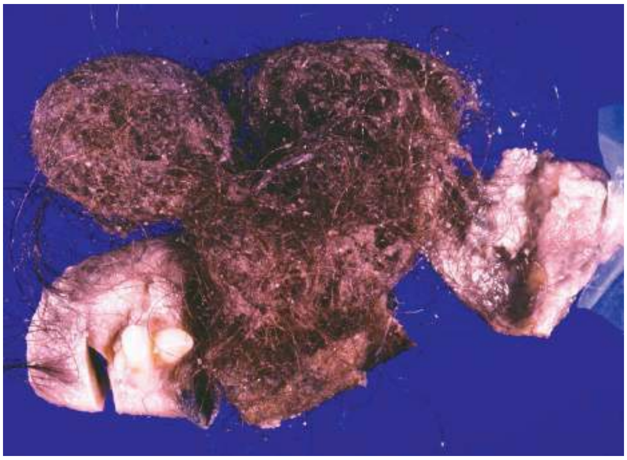

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Figure 24-3. Teratoma of Testes, Producing Hair and Teeth

Mixed germ cell tumors. As many as 60% of germ cell tumors contain >1 component. When both teratoma and embryonal carcinoma are present, the name *teratocarcinoma* is used.

Sex Cord–Stromal Tumors

Sex cord–stromal tumors include Leydig cell and Sertoli cell tumors.

- **Leydig cell tumors** cause painless testicular masses, and have a bimodal distribution (prepubertal and age >50). They may produce androgens and estrogens.
  - In adults, the hormonal secretion can produce gynecomastia; in children, it can produce precocious puberty.
  - Benign tumors (90%) have an excellent prognosis; malignant tumors (10%) can be refractory to chemotherapy and radiation therapy.
  - Tumor cells have abundant pink cytoplasm.
- **Sertoli cell tumors** are rare and usually benign. Microscopically, they show tubule formation.

Other Tumors

- **Testicular lymphoma** is the most common testicular tumor in men >age 60. It is most commonly non-Hodgkin lymphoma, diffuse large cell type.
- **Scrotal squamous cell carcinoma** is associated with exposure to soot (chimney sweeps).

PROSTATE

Benign prostatic hyperplasia (BPH) (also called nodular hyperplasia; glandular and stromal hyperplasia) is extremely common. Androgens (dihydrotestosterone) play an important role in the pathogenesis, and the lesion is not premalignant. The incidence increases with age (age 60 is 70%; age 70 is 80%). BPH typically presents with the following:

- Decreased caliber and force of stream
- Trouble starting (hesitancy)/stopping the stream
- Postvoid dribbling
- Urinary retention
- Incontinence
- Urgency/frequency
- Nocturia/dysuria
- Possible elevation in prostate specific antigen (PSA) but usually <10 ng/mL

Complications include urinary tract infection, urinary bladder trabeculation and diverticula formation, and hydronephrosis and renal failure (rare). Treatment varies, with available modalities including transurethral resection of prostate (TURP); the 5-alpha reductase inhibitor, finasteride (Proscar); and the selective alpaha-1 receptor blockers, terazosin and prazosin.

Grossly, BPH causes an enlarged prostate with well-demarcated nodules in the transition and central (periurethral) zones, which often results in slit-like compression of the prostatic urethra. Microscopically, the lesion shows glandular and stromal hyperplasia resulting in the characteristic prostate enlargement.

Prostate adenocarcinoma is the most common cancer in men in the United States and the second most common cause of cancer death in men. The incidence increases with age, and the highest rate is in African Americans.

- Often clinically silent but may present with lower back pain secondary to metastasis
- Advanced localized disease may present with urinary tract obstruction or UTI (rare)
- Tumor can be detected with digital rectal exam (induration), serum PSA level, and transrectal U/S and biopsy
- Metastases most commonly involve obturator and pelvic lymph nodes
- Osteoblastic bone metastasis to lumbar spine can occur, and can be associated with elevated alkaline phosphatase

Bridge to Anatomy

- Prostatic hyperplasia → transitional/periurethral zone
- Prostatic carcinoma → peripheral zone

Treatment of local disease is prostatectomy and/or external beam radiation. Metastatic disease is treated with orchiectomy, estrogens, or LHRH agonists and anti-androgens. The disease course can be monitored with PSA levels.

Pathologically, an ill-defined, firm, yellow mass commonly arises in the posterior aspect of the peripheral zone. Microscopically, adenocarcinoma is graded with the **Gleason system**, which scores glandular differentiation. *TMPRSS2-ETS* fusion genes are present in nearly 50% of all prostatic carcinomas.

Prostatitis

- Acute prostatitis is usually caused by intraprostatic reflux of urinary tract pathogens.
- Chronic prostatitis may develop following recurrent acute prostatitis, and bacterial pathogens may not be detectable. Chronic nonbacterial prostatitis (**chronic pelvic pain syndrome**) is more common than bacterial prostatitis.
- Clinical findings can include fever (acute prostatitis), pain (lower back, perineal, or suprapubic), painful prostate on rectal exam, and dysuria (with possible hematuria).

Endocrine Pathology 25

Learning Objectives

- ❑ Demonstrate understanding of disease of the thyroid, parathyroid, adrenal, pituitary, and pineal gland
- ❑ Describe the relationship between the hypothalamus and pituitary gland
- ❑ Solve problems concerning multiple endocrine neoplasia syndromes
- ❑ Explain information related to diabetes mellitus

THYROID GLAND

Multinodular goiter (nontoxic goiter) refers to an enlarged thyroid gland with multiple colloid nodules. Females are affected more often than males. It is frequently asymptomatic, and the patient is typically euthyroid, with normal T4, T3, and TSH. **Plummer syndrome** is the development of hyperthyroidism (toxic multinodular goiter) late in the course.

Microscopically, the tissue shows nodules of varying sizes composed of colloid follicles. Calcification, hemorrhage, cystic degeneration, and fibrosis can also be present.

Hyperthyroidism

The term ***hyperthyroidism*** is used when the mean metabolic rate of all cells is increased due to increased T4 or T3. Clinical features include tachycardia and palpitations; nervousness and diaphoresis; heat intolerance; weakness and tremors; diarrhea; and weight loss despite a good appetite. Lab studies show elevated free T4.

- In primary hyperthyroidism, TSH is decreased.
- In secondary and tertiary hyperthyroidism, TSH is elevated.

Graves disease is an autoimmune disease characterized by production of IgG autoantibodies to the TSH receptor. Females are affected more frequently than males, with peak age 20–40. Clinical features include hyperthyroidism, diffuse goiter, ophthalmopathy (exophthalmus), and dermopathy (pretibial myxedema). Microscopically, the thyroid has hyperplastic follicles with scalloped colloid.

Other causes of hyperthyroidism include toxic multinodular goiter, toxic adenoma (functioning adenoma producing thyroid hormone), and Hashimoto and subacute thyroiditis (transient hyperthyroidism).

Clinical Correlate

TSH is the most sensitive test in thyroid disease. If it is normal, the patient is euthyroid.

Hypothyroidism

The term ***hypothyroidism*** is used when the mean metabolic rate of all cells is decreased due to decreased T4 or T3. Clinical features include fatigue and lethargy; sensitivity to cold temperatures; decreased cardiac output; myxedema (accumulation of proteoglycans and water); facial and periorbital edema; peripheral edema of the hands and feet; deep voice; macroglossia; constipation; and anovulatory cycles. Lab studies show decreased free T4.

- In primary hypothyroidism, TSH is elevated.
- In secondary and tertiary hypothyroidism, TSH is decreased.

Iatrogenic hypothyroidism is the most common cause of hypothyroidism in the United States, and is secondary to thyroidectomy or radioactive iodine treatment. Treatment is thyroid hormone replacement.

Congenital hypothyroidism (cretinism) in endemic regions is due to iodine deficiency during intrauterine and neonatal life, and in nonendemic regions is due to thyroid dysgenesis. Patients present with failure to thrive, stunted bone growth and dwarfism, spasticity and motor incoordination, and mental retardation. Goiter is seen in endemic cretinism.

Endemic goiter is due to dietary deficiency of iodine; it is uncommon in the United States.

Note

The original name for the autoantibodies of Graves disease was long-acting thyroid stimulator (LATS). The current name is **thyroid-stimulating immunoglobulin (TSI)**.

Thyroiditis

Hashimoto thyroiditis is a chronic autoimmune disease characterized by immune destruction of the thyroid gland and hypothyroidism. It is the most common noniatrogenic/nonidiopathic cause of hypothyroidism in the United States; it most commonly causes painless goiter in females more than males, with peak age 40–65.

Hashimoto thyroiditis is the most common cause of hypothyroidism (due to destruction of thyroid tissue), though the initial inflammation may cause transient hyperthyroidism (hashitoxicosis). Hashimoto may be associated with other autoimmune diseases (SLE, rheumatoid arthritis, Sjögren syndrome, etc.), and it has an increased risk of non-Hodgkin B-cell lymphoma. Grossly, Hashimoto produces a pale, enlarged thyroid gland; microscopically, it shows lymphocytic inflammation with germinal centers and epithelial “Hürthle cell” changes.

Subacute thyroiditis (also called de Quervain thyroiditis and granulomatous thyroiditis) is the second most common form of thyroiditis; it affects females more than males, with peak age 30–50. Patients may complain of odynophagia (pain on swallowing).

- Typically preceded by a viral illness
- Produces a tender, firm, enlarged thyroid gland
- May be accompanied by transient hyperthyroidism

Microscopy shows granulomatous thyroiditis. The disease typically follows a self-limited course.

Riedel thyroiditis is a rare disease of unknown etiology, characterized by destruction of the thyroid gland by dense fibrosis and fibrosis of surrounding structures (trachea and esophagus). It affects females more than males, and most patients are middle-aged.

- Causes an irregular, hard thyroid that is adherent to adjacent structures
- May clinically mimic carcinoma and present with stridor, dyspnea, or dysphagia

Microscopic exam shows dense fibrous replacement of the thyroid gland with chronic inflammation. Reidel thyroiditis is associated with retroperitoneal and mediastinal fibrosis.

Thyroid Neoplasia

Thyroglossal duct cyst presents as a midline neck mass in a young patient. Its epithelium varies with location (squamous/respiratory). It may become infected and painful. Treatment is surgical.

Adenomas: follicular adenoma is the most common. Clinically, adenomas are usually painless, solitary, encapsulated nodules that appear “cold” on thyroid scans. They may be functional and cause hyperthyroidism (toxic adenoma).

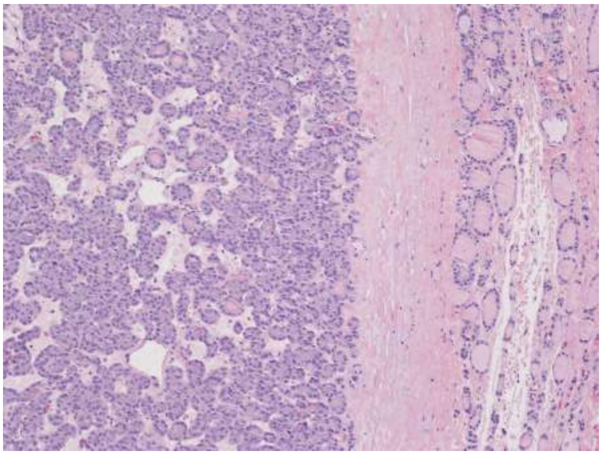

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Figure 25-1. Follicular Adenoma *(left)*, Separated from Normal Thyroid Parenchyma *(right)*, by the Capsule *(center)*

Papillary carcinoma accounts for 80% of malignant thyroid tumors. It affects females more than males, with peak age 20–50. Radiation exposure is a risk factor. Resection is curative in most cases. Radiotherapy with iodine 131 is effective for metastases. The prognosis is excellent, with 20-year survival 90% due to slow growth and metastasis to regional cervical lymph nodes. There are chromosomal rearrangements of the *RET* oncogene.

Microscopically, the tumor typically exhibits a papillary pattern. Occasional psammoma bodies may be seen. Characteristic nuclear features include clear “Orphan Annie eye” nuclei, nuclear grooves, and intranuclear cytoplasmic inclusions. Lymphatic spread to cervical nodes is common.

Follicular carcinoma accounts for 15% of malignant thyroid tumors. It affects females more than males, with peak age 40–60. Hematogenous metastasis to the bones or lungs is common. These cancers are microscopically distinguished from follicular adenoma by the presence of capsular invasion.

Medullary carcinoma accounts for 5% of malignant thyroid tumors. It arises from C cells (parafollicular cells) and secretes calcitonin. Microscopic exam shows nests of polygonal cells in an amyloid stroma. A minority of cases (25%) is associated with MEN 2 and MEN 3 syndromes, and those cases tend to be multicentric. Activating *RET* mutations are present in familial and sporadic types.

Anaplastic carcinoma affects females more than males, with peak age >60. It can present with a firm, enlarging, and bulky mass, or with dyspnea and dysphagia. The tumor has a tendency for early widespread metastasis and invasion of the trachea and esophagus. Microscopically, the tumor is composed of undifferentiated, anaplastic, and pleomorphic cells. This very aggressive tumor is often rapidly fatal.

PARATHYROID GLANDS

Primary hyperparathyroidism can be caused by the following:

- Adenomas (80%); may be associated with MEN 1
- Parathyroid hyperplasia (15%)
    - Characterized by diffuse enlargement of all 4 glands; the enlarged glands are usually composed of chief cells
    - Parathyroid carcinoma is very rare
- Hyperparathyroidism can also occur as a paraneoplastic syndrome of lung and renal cell carcinomas.

The excess production of parathyroid hormone (PTH) leads to hypercalcemia, with lab studies showing elevated serum calcium and PTH.

Primary hyperparathyroidism is often asymptomatic, but may cause kidney stones, osteoporosis and osteitis fibrosa cystica, metastatic calcifications, or neurologic changes.

Secondary hyperparathyroidism is caused by any disease that results in hypocalcemia, leading to increased secretion of PTH by the parathyroid glands; it can result from chronic renal failure, vitamin D deficiency, or malabsorption.

Hypoparathyroidism can result from the surgical removal of glands during thyroidectomy, DiGeorge syndrome, or a hereditary autoimmune syndrome caused by mutations in the autoimmune regulator gene *AIRE*. Patients present with muscle spasm and tingling of toes and lips. The hypocalcemia may also cause psychiatric disturbances and cardiac conduction defects (ECG: prolonged QT interval).

Treatment of hypoparathyroidism is vitamin D and calcium.

Note

Osteitis fibrosa cystica (von Recklinghausen disease) is seen when excessive parathyroid hormone (hyperparathyroidism) causes osteoclast activation and generalized bone resorption (causing possible bone pain, deformities, and fractures). It is common in primary hyperparathyroidism.

- Excess parathyroid hormone may be produced by parathyroid adenoma or parathyroid hyperplasia
- “Brown tumors” are masses produced by cystic enlargement of bones with areas of fibrosis and organized hemorrhage

PITUITARY GLAND, HYPOTHALAMUS, AND PINEAL GLAND

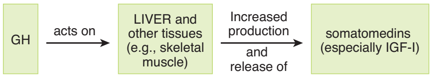
Pituitary adenomas are categorized as follows:

- **Microadenoma** if <**1 cm**
- **Macroadenoma** if >**1 cm**

Macroadenomas cause visual field defects.

GNAS1 mutations are common. Pathologically, adenomas are monomorphic compared with normal pituitary.

- **Prolactinoma** is the most common type of pituitary adenoma. Lactotroph cells secrete prolactin, which results in hyperprolactinemia. Clinical features include galactorrhea, amenorrhea, and infertility, or decreased libido and impotence.
- **Nonfunctional adenoma** may produce hypopituitarism.
- **Growth-hormone–producing adenoma** is characterized by elevated growth hormone (GH) and elevated somatomedin C (insulin-like growth factor 1 [IGF-1]). It causes gigantism in children and acromegaly in adults.



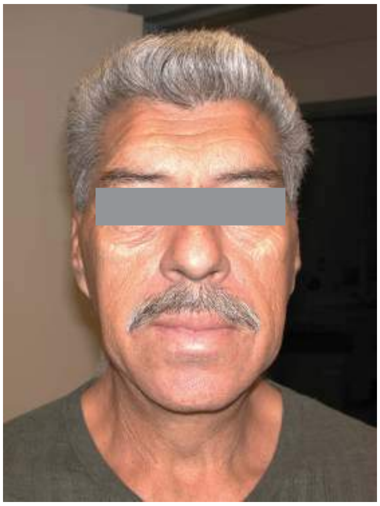


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Figure 25-2. Coarse Facial Features and Protruding Jaw Seen with Acromegaly

Sheehan syndrome is ischemic necrosis of the pituitary secondary to hypotension from postpartum hemorrhage resulting in panhypopituitarism.

Clinical Correlate

Any pituitary tumor that destroys >75% of the pituitary may result in panhypopituitarism, which is characterized by abnormalities of the thyroid, adrenal gland, and reproductive organs. Common causes are:

- Pituitary adenomas
- Sheehan syndrome
- Craniopharyngiomas

Posterior pituitary syndromes include the following:

- **Diabetes insipidus**
    - Central diabetes insipidus is caused by ADH deficiency, which results in hypotonic polyuria, polydipsia, hypernatremia, and dehydration. Causes include head trauma and tumors.
    - Nephrogenic diabetes insipidus is caused by a lack of renal response to ADH.
- **Syndrome of inappropriate ADH secretion (SIADH)** is caused by excessive production of antidiuretic hormone (ADH), resulting in oliguria, water retention, hyponatremia, and cerebral edema. Causes include paraneoplastic syndrome and head trauma.

Craniopharyngioma is a benign pituitary tumor derived from Rathke pouch remnants that are usually located above the sella turcica, but can extend downward to destroy the pituitary. It is the most common cause of hypopituitarism in children.

Hypothalamic disorders can cause a variety of problems:

- **Hypopituitarism** (including dwarfism) can be due to a lack of releasing hormones from the hypothalamus.
- **Central diabetes insipidus** is due to lack of ADH synthesis.
- **Precocious puberty** is usually due to a midline hamartoma in boys.
- The hypothalamus can also be affected in **hydrocephalus**.
- **Visual field changes** can complicate hypothalamic disorders.
- Masses can affect the hypothalamus, i.e., pituitary adenoma, craniopharyngioma, midline hamartoma, and Langerhans histiocytosis.
- Inflammatory processes can affect the hypothalamus, i.e., sarcoidosis and meningitis.

Pineal diseases include dystrophic calcification (a useful landmark for radiologists) and rarely tumors, with most being germ cell tumors.

Clinical Correlate

The most common cause of ectopic ADH secretion is cancer (especially small cell lung cancer, though any lung lesion can lead to this manifestation). For this reason, SIADH may be a marker for occult malignancy. Drugs that may also lead to SIADH include chlorpropamide, carbamazepine, cyclophosphamide, tricyclic antidepressants, and SSRIs.

ADRENAL GLAND

Cushing syndrome is characterized by increased levels of glucocorticoids. It is investigated with lab and imaging studies (*see* Physiology Lecture Notes). The most common cause is exogenous glucocorticoid administration.

Endogenous causes include:

- Cushing disease (hypersecretion of ACTH, usually due to a pituitary microadenoma)
- Secretion of ACTH from nonpituitary tumors (e.g., small cell lung cancer)
- ACTH-independent Cushing syndrome due to adrenal neoplasia

Clinical manifestations include hypertension, weight gain (truncal obesity, "buffalo hump" and moon facies), cutaneous striae, hirsutism and mental disturbances.

Note

Administration of **dexamethasone** (a cortisol analog) will typically suppress pituitary ACTH production, resulting in suppression of adrenal cortisol production and a decrease in urinary free cortisol.

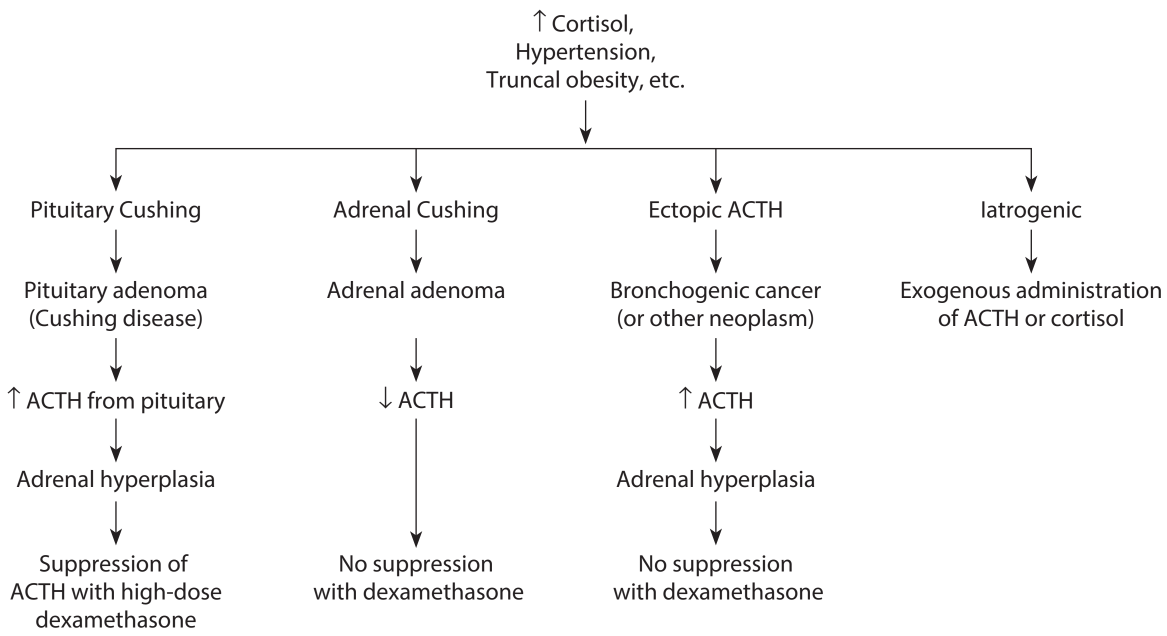

Figure 25-3. Cushing Syndrome and Its Effects

Hyperaldosteronism may cause hypertension and hypokalemia.

- **Primary** (decreased plasma renin)
  - Adrenocortical neoplasm: adenoma (Conn syndrome)
    - Circumscribed yellow nodule with vacuolated cells
    - Carcinoma: poorly demarcated lesion with cystic change and pleomorphic cells
  - Bilateral nodular hyperplasia of the adrenal
- **Secondary** (increased plasma renin) (e.g., renal artery stenosis)

Adrenogenital syndromes are adrenal disorders characterized by excess production of androgens and virilization. They are caused either by adrenocortical adenoma/carcinoma, which produces androgens, or by congenital adrenal hyperplasia, a cluster of autosomal recessive enzyme defects (most common is 21-hydroxylase deficiency).

Waterhouse-Friderichsen syndrome (acute adrenal insufficiency) is a potentially fatal, bilateral hemorrhagic infarction of the adrenal glands associated with sepsis, often due to a *N. meningitidis* infection in children. It is clinically characterized by disseminated intravascular coagulation (DIC), acute respiratory distress syndrome, hypotension and shock, and acute adrenal insufficiency. Treatment is antibiotics and steroid replacement.

Addison's disease (chronic adrenocortical insufficiency) is caused by destruction of the adrenal cortex, leading to a deficiency of glucocorticoids, mineralocorticoids, and androgens. The most common cause is autoimmune adrenalitis, though adrenal involvement by TB or metastatic cancer are other possible causes. Patients present with gradual onset of weakness, skin hyperpigmentation, hypotension, hypoglycemia, poor response to stress, and loss of libido. Treatment is steroid replacement.

Bridge to Embryology

- The cells of the **adrenal medulla** are derived from neural crest cells.
- The cells of the **adrenal cortex** are derived from mesoderm.

Secondary adrenocortical insufficiency may be caused by disorders of the hypothalamus or pituitary (cancer or infection, for example). Since ACTH levels are low, hyperpigmentation does not occur.

Pheochromocytoma ("dark/dusky-colored tumor") is an uncommon benign tumor of the adrenal medulla, which produces catecholamines (norepinephrine and epinephrine). It can present with sustained or episodic hypertension and associated severe headache, tachycardia, palpitations, diaphoresis, and anxiety. Note the **rule of 10s**:

10% occur in **children**

- 10% are **bilateral**
- 10% are **malignant**
- 10% are **familial (MEN 2A and 2B)**
- 10% occur **outside the adrenal gland**

Urinary vanillylmandelic acid (VMA) and catecholamines are elevated. Microscopically, the tumor shows nests of cells (Zellballen) with abundant cytoplasm. Treatment is control of the patient's BP and surgical removal of the tumor.

MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES

Multiple endocrine neoplasia (MEN) syndromes are autosomal dominant conditions with incomplete penetrance that are characterized by hyperplasia and tumors of endocrine glands occurring at a young age.

- **MEN 1** (Werner syndrome) features tumors of the pituitary gland, parathyroids, and pancreas.
- Associated with peptic ulcers and Zollinger-Ellison syndrome
    - Affected gene is *MEN1*, a tumor suppressor gene which encodes a nuclear protein called menin
- **MEN 2A** (Sipple syndrome) features medullary carcinoma of the thyroid, pheochromocytoma, and parathyroid hyperplasia or adenoma.
    - Mutation of *RET* proto-oncogene
- **MEN 2B** features medullary carcinoma of the thyroid, pheochromocytoma, and mucocutaneous neuromas.
- Mutation of *RET* proto-oncogene

DIABETES MELLITUS

Diabetes mellitus (DM) is a chronic systemic disease characterized by insulin deficiency or peripheral resistance, resulting in hyperglycemia and nonenzymatic glycosylation of proteins. Diagnosis is established by demonstrating **either** of the following:

- Fasting glucose >126 mg/dL on at least 2 separate occasions
- Positive glucose tolerance test

A glycated hemoglobin (HbA1c) assay is used for certain patient populations. The diagnostic cutoff is 6.5%.

Type 1 diabetes (T1D) represents 10% of cases of diabetes.

- Affects children and adolescents, usually age <20
- Risk factors include Northern European ancestry and specific HLA types (DR3, DR4, and DQ)
- Pathogenesis is a lack of insulin due to autoimmune destruction of β cells (type IV hypersensitivity reaction)
- Patients are absolutely dependent on insulin to prevent ketoacidosis and coma
- Thought to be caused by autoimmune reaction triggered by an infection (Coxsackie B virus) in a genetically susceptible individual

T1D can present with polydipsia, polyuria, and polyphagia; dehydration and electrolyte imbalance; metabolic ketoacidosis; and coma and potentially death. Microscopically, lymphocytic inflammation involves the islets of Langerhans (insulitis), leading to loss of β cells and fibrosis of the islets. Treatment is insulin.

Type 2 diabetes (T2D) represents 90% of cases.

- Affects obese individuals, both children and adults
- Approximately 10 million people in the United States are affected (half are undiagnosed), and incidence increases with age
- Risk factors include obesity, increasing age, and genetic predisposition
- Pathogenesis involves relatively reduced insulin secretion; *peripheral insulin resistance* is the term used for reduced tissue sensitivity to insulin due to decreased numbers of insulin receptors on the cell membranes.

T2D is often asymptomatic, but it can present with either polydipsia, polyuria, and polyphagia, or with hyperosmolar nonketotic diabetic coma. Microscopically, the changes are nonspecific, and can include focal atrophy and amyloid deposition in islets (hyalinization). Treatment is diet/weight loss, oral antidiabetic drugs, and insulin as needed (more common in long-standing cases).

Vascular pathology. Diabetes is a major risk factor for atherosclerosis and its complications, including myocardial infarction (most common cause of death), stroke (CVA), and peripheral vascular disease. The vascular disease can lead to atrophy of skin and loss of hair of the lower extremities, claudication, nonhealing ulcers, and gangrene of lower extremities.

Diabetic nephropathy includes glomerular lesions, arteriolosclerosis, and pyelonephritis (*see* Renal chapter).

Diabetic retinopathy. Nonproliferative retinopathy is characterized by microaneurysms, retinal hemorrhages, and retinal exudates. Proliferative retinopathy is characterized by neovascularization and fibrosis. Diabetics also have an increased incidence of cataracts and glaucoma.

Diabetic neuropathy can cause peripheral neuropathy, neurogenic bladder, and sexual impotence.

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Figure 25-4. Diabetic Retinopathy with Hemorrhages, Venous Beading, and Looping of Vessels

Bone Pathology 26

Learning Objectives

- ❑ Describe normal bone
- ❑ Explain information related to hereditary bone disorders
- ❑ Answer questions about Paget disease
- ❑ Differentiate osteoporosis, osteomalacia, and rickets
- ❑ Solve problems concerning osteomyelitis, benign tumors of bone, and malignant tumors of bone

NORMAL BONE

Normal bone is composed of organic matrix and inorganic matrix.

- The **organic matrix** includes cells, type I collagen (90% of bone protein), osteocalcin, glycoproteins, and proteoglycans.
- The **inorganic matrix** includes calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, magnesium, potassium, chloride, sodium, and fluoride.

There are 3 cell types.

- **Osteoblasts** are responsible for the production of osteoid (unmineralized bone); they contain high amounts of alkaline phosphatase, have receptors for parathyroid hormone (PTH), and modulate osteoclast function.
- **Osteocytes** are responsible for bone maintenance; they are osteoblasts that have become incorporated in the matrix.
- **Osteoclasts** are responsible for bone resorption; they contain high amounts of acid phosphatase and collagenase, and resorb bone within Howship's lacunae.

Bone remodeling occurs throughout life and is necessary to maintain healthy bones. Bone resorption by osteoclasts is tightly balanced with bone formation by osteoblasts.

Important hormones involved in bone physiology include parathyroid hormone (PTH), calcitonin, vitamin D, estrogen, thyroid hormone, cortisol, and growth hormone.

Formation of bones is as follows:

- **Intramembranous bone** occurs as direct bone formation without a "cartilage model." Intramembranous bones include flat bones such as the cranium, clavicle, vertebrae, wrist, and ankle bones. Intramembranous growth is also involved in appositional bone growth.
- **Endochondral bone** is indirect bone formation from a "cartilage model" at the epiphyseal growth plates; this type of bone formation occurs in long bones such as the femur, humerus, tibia, fibula, etc.

HEREDITARY BONE DISORDERS

Achondroplasia is the most common form of inherited dwarfism. It is caused by an autosomal dominant mutation in fibroblast growth factor receptor 3 (*FGFR3*). Activation of *FGFR3* inhibits cartilage synthesis at the epiphyseal growth plate, resulting in decreased endochondral bone formation and premature ossification of the growth plates.

- Long bones are short and thick, leading to dwarfism with short extremities.
- Cranial and vertebral bones are spared, leading to relatively large head and trunk.
- Intelligence, life span, and reproductive ability are normal.

Osteogenesis imperfecta (OI) ("brittle bone disease") is a hereditary defect leading to abnormal synthesis of type I collagen.

- Patients have generalized osteopenia (brittle bones), resulting in recurrent fractures and skeletal deformity
- Abnormally thin sclera with blue hue is common
- Laxity of joint ligaments leads to hypermobility
- Involvement of inner and middle ear bones produces deafness
- Occasional dentinogenesis imperfecta, characterized by small, fragile, and discolored teeth due to a deficiency of dentin
- Dermis may be abnormally thin, and skin is susceptible to easy bruising
- Treatment is supportive

Osteopetrosis (or marble bone disease) is a hereditary defect leading to decreased osteoclast function, with resulting decreased resorption and thick sclerotic bones. X-ray shows symmetrical generalized osteosclerosis. Long bones may have broadened metaphyses, causing an "Erlenmeyer flask"-shaped deformity. Treatment is hematopoietic stem cell transplantation.

- **Autosomal recessive** type (**malignant**):
  - Affects infants and children (causes multiple fractures and early death due to anemia, infection, and hemorrhage)
- **Autosomal dominant** type (**benign**):
  - Affects adults (causes fractures, mild anemia, and cranial nerve impingement)

Pathology shows increased bone density and thickening of bone cortex. Myelophthisic anemia may result from marrow crowding. Cranial nerve compression due to narrowing of cranial foramina may result in blindness, deafness, and facial nerve palsies. Hydrocephalus may develop due to obstruction of CSF.

PAGET DISEASE

Paget disease (osteitis deformans) is a localized disorder of bone remodeling, resulting in excessive bone resorption followed by disorganized bone replacement, producing thickened but weak bone that is susceptible to deformity and fracture. There is an association with paramyxovirus and mutations of *SQSTM1*.

- Seen in those age >40
- Common in those of European ancestry
- Common sites of involvement include the skull, pelvis, femur, and vertebrae
- Majority of cases are polyostotic and mild

Paget disease develops in 3 stages:

- **Osteolytic stage** (osteoclastic activity predominates)
- **Mixed osteolytic-osteoblastic stage**
- **Osteosclerotic stage** (osteoblastic activity predominates in this "burnout stage")

Paget disease can cause bone pain and deformity, fractures, and warmth of the overlying skin due to bone hypervascularity. X-rays show bone enlargement with lytic and sclerotic areas. Lab studies show highly elevated serum alkaline phosphatase and increased levels of urinary hydroxyproline. Complications include arteriovenous shunts within marrow, which may result in high-output cardiac failure and an increased incidence of osteosarcoma and other sarcomas.

Microscopically, there is a haphazard arrangement of cement lines, creating a "mosaic pattern" of lamellar bone. Involved bones are thick but weak and fracture easily. Skull involvement leads to increased head size and foraminal narrowing that can impinge on cranial nerves, often leading to deafness. Involvement of facial bones may produce a lion-like facies.

OSTEOPOROSIS

Osteoporosis is decreased bone mass (osteopenia), resulting in thin, fragile bones susceptible to fracture. It is the most common bone disorder in the United States. It most commonly occurs in postmenopausal Caucasian women and the elderly.

Note

In osteoporosis, bone is formed normally but in decreased amounts.

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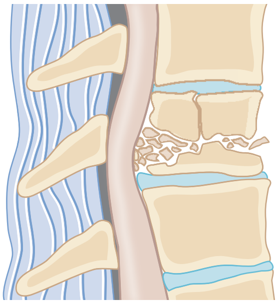
Figure 26-1. Marked Thinning of Bony Trabeculae in Osteoporosis

Primary causes of osteoporosis include the following:

- Estrogen deficiency (postmenopausal, Turner syndrome)
- Genetic factors (low density of original bone)
- Lack of exercise
- Old age
- Nutritional factors

Secondary causes include immobilization, endocrinopathies (e.g., Cushing disease, thyrotoxicosis), malnutrition (e.g., deficiencies of calcium, vitamins C and D, protein), corticosteroids, smoking/alcohol consumption, genetic disease (e.g., Gaucher disease).

- Patients may experience bone pain and fractures; weight-bearing bones are predisposed to fractures.
- Common fracture sites include vertebrae (compression fracture); femoral neck (hip fracture); and distal radius (Colles fracture).
- Kyphosis and loss of height may result.
- X-rays show generalized radiolucency of bone (osteopenia).



Compression Fracture

Dual-energy x-ray absorptiometry (DEXA) can measure bone mineral density to predict fracture risk. Lab studies may show normal serum calcium, phosphorus, and alkaline phosphatase, but the diagnosis is not based on labs. Microscopically, the bone has thinned cortical and trabecular bone.

Treatment can include estrogen replacement therapy (controversial; not recommended currently); weight-bearing exercise; calcium and vitamin D; bisphosphonate (alendronate); and calcitonin.

OSTEOMALACIA AND RICKETS

Osteomalacia and **rickets** are both characterized by decreased mineralization of newly formed bone. They are usually caused by deficiency or abnormal metabolism of vitamin D. Specific causes include dietary deficiency of vitamin D, intestinal malabsorption, lack of sunlight, and renal and liver disease. Treatment is vitamin D and calcium supplementation.

Osteomalacia (adults) is due to impaired mineralization of the osteoid matrix resulting in thin, fragile bones susceptible to fracture. The patient may present clinically with bone pain or fractures (vertebrae, hips, and wrist). X-rays show transverse lucencies called Looser zones. Lab studies show low serum calcium, low serum phosphorus, and high alkaline phosphatase.

Rickets (children) occurs in children prior to closure of the epiphyses. Both remodeled bone and bone formed at the epiphyseal growth plate are undermineralized. Enchondral bone formation is affected, leading to skeletal deformities. Skull deformities include craniotabes (softening, seen in early infancy) and frontal bossing (hardening, later in childhood). The "rachitic rosary" is a deformity of the chest wall as a result of an overgrowth of cartilage at the costochondral junction. Pectus carinatum, lumbar lordosis, bowing of the legs, and fractures also occur.

Note

Rickets and osteomalacia are disorders of osteoid mineralization; osteoid is produced in normal amounts but is not calcified properly.

Clinical Correlate

Lab findings help to distinguish osteomalacia from osteoporosis.

OSTEOMYELITIS

Pyogenic osteomyelitis is bone inflammation due to bacterial infection. The most common route of infection is **hematogenous spread** (leading to seeding of bone after bacteremia); this type of spread often affects the metaphysis. Other routes of spread include direct inoculation (e.g., trauma) and spread from an adjacent site of infection (e.g., prosthetic joint).

The most common infecting organism is *S. aureus*; other important pathogens include *E. coli*, streptococci, gonococci, *H. influenzae*, *Salmonella* (common in sickle cell disease), and *Pseudomonas* (common in IV drug abusers and diabetics).

Clinically, osteomyelitis is characterized by fever and leukocytosis; and localized pain, erythema, and swelling. X-ray studies may be normal for up to 2 weeks, and then initially show periosteal elevation followed by a possible lytic focus with surrounding sclerosis. MRI and nuclear medicine studies are more sensitive and specific.

Microscopic examination shows suppurative inflammation. Vascular insufficiency can lead to ischemic necrosis of bone; a sequestrum is an area of necrotic bone, while an involucrum is the new bone formation that surrounds the sequestrum. The diagnosis is established with blood cultures or with bone biopsy and culture.

Patients are treated with antibiotics and some may require surgical drainage. Complications include fracture, intraosseous (Brodie) abscess, secondary amyloidosis, sinus tract formation, squamous cell carcinoma of the skin at the site of a persistent draining sinus tract, and, rarely, osteogenic sarcoma.

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Figure 26-2. Tibial Radiolucencies Representing Osteomyelitis.

Tuberculous osteomyelitis is seen in 1% of cases of TB. It presents with pain or tenderness, fever, night sweats, and weight loss. Biopsy shows caseating granulomas with extensive destruction of the bones. Common sites of involvement include thoracic and lumbar vertebrae ("Pott disease"). Complications include vertebral compression fracture, psoas abscesses, and secondary amyloidosis.

MISCELLANEOUS BONE DISORDERS

Avascular necrosis (or aseptic necrosis and osteonecrosis) is the term used for ischemic necrosis of bone and bone marrow. Causes include trauma and/or fracture (most common); idiopathic; steroid use; sickle cell anemia; Gaucher disease; and caisson disease. Avascular necrosis can be complicated by osteoarthritis and fractures.

Osteitis fibrosa cystica (or von Recklinghausen disease of bone) is seen when excessive parathyroid hormone (hyperparathyroidism) causes osteoclast activation and generalized bone resorption, resulting in possible bone pain, bone deformities, and fractures.

- Seen commonly in primary hyperparathyroidism
- Excess parathyroid hormone may be produced by parathyroid adenoma or parathyroid hyperplasia
- Can be resolved if hyperparathyroidism is treated

Microscopic exam shows excess bone resorption with increased number of osteoclasts, fibrous replacement of marrow, and cystic spaces in trabecular bone (dissecting osteitis). "Brown tumors" are brown bone masses produced by cystic enlargement of bones with areas of fibrosis and organized hemorrhage.

Hypertrophic osteoarthropathy presents with painful swelling of wrists, fingers, ankles, knees, or elbows.

- Seen in the setting of bronchogenic carcinoma (a paraneoplastic syndrome), chronic lung diseases, cyanotic congenital heart disease, and inflammatory bowel disease
- Can regress if underlying disease is treated

Pathologically, the ends of long bones show periosteal new bone formation, which can produce digital clubbing and often arthritis of adjacent joints.

Osgood-Schlatter disease is a common cause of knee pain in adolescents. It develops when stress from the quadriceps during rapid growth causes inflammation of the proximal tibial apophysis at the insertion of the patellar tendon. Permanent changes to the knees (knobby knees) may develop. The lesion is not usually biopsied.

Fibrous dysplasia presents with painful swelling, deformity, or pathologic fracture of involved bone (typically ribs, femur, or cranial bones), usually in children and young adults. *GNAS* gene mutations cause osteoblasts to produce fibrous tissue (microscopically, irregularly scattered trabeculae) rather than bone.

Note

McCune-Albright syndrome presents with café au lait spots, precocious puberty (and other endocrine abnormalities), and polyostotic fibrous dysplasia.

BENIGN TUMORS OF BONE

Osteoma is a benign neoplasm that frequently involves the skull and facial bones. Osteoma can be associated with Gardner syndrome. Malignant transformation doesn't occur.

Osteoid osteoma is a benign, painful growth of the diaphysis of a long bone, often the tibia or femur. Males are affected more than females, with peak age 5–25. Pain tends to be worse at night and relieved by aspirin. X-rays show central radiolucency surrounded by a sclerotic rim. Microscopically, osteoblasts line randomly connected trabeculae.

Osteoblastoma is similar to an osteoid osteoma but larger (>2 cm) and often involves vertebrae.

Osteochondroma (exostosis) is a benign bony metaphyseal growth capped with cartilage, which originates from epiphyseal growth plate. It typically presents in adolescent males who have firm, solitary growths at the ends of long bones. It may be asymptomatic, cause pain, produce deformity, or undergo malignant transformation (rare). *Osteochondromatosis* (multiple hereditary exostoses) produces multiple, often symmetric, osteochondromas. Excision is usually curative.

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Figure 26-3. Osteochondroma Seen on Bony Protuberance on Distal Ulna

Enchondroma is a benign cartilaginous growth within the medullary cavity of bone, usually involving the hands and feet. It is typically solitary, asymptomatic, and requires no treatment. Multiple enchondromas (*enchondromatosis*) can occur as part of both Ollier disease (multiple and unilateral) and Maffucci syndrome (with hemangiomas), and carry a risk of malignant transformation.

Giant cell tumor of bone ("osteoclastoma") is a benign neoplasm containing multinucleated giant cells admixed with stromal cells. It is uncommon but occurs more often in females than in males, with peak age 20–50. Clinically, the tumor produces a bulky mass with pain and fractures. X-rays show an expanding lytic lesion surrounded by a thin rim of bone, with a possible "soap bubble" appearance. Treatment is surgery (curettage or *en bloc* resection). Osteoclastoma is locally aggressive with a high rate of recurrence (40–60%). In spite of being considered benign, approximately 2% will metastasize to the lungs.

Grossly, the tumor causes a red-brown mass with cystic degeneration that often involves the epiphyses of long bones, usually around the knee (distal femur and proximal tibia). Microscopically, multiple osteoclast-like giant cells are distributed within a background of mononuclear stromal cells.

MALIGNANT TUMORS OF BONE

Osteosarcoma (osteogenic sarcoma) is the most common primary malignant tumor of bone. It occurs more frequently in males than in females, with most cases in teenage years (ages 10–25). Patients with familial retinoblastoma have a high risk.

Osteosarcoma presents with localized pain and swelling. The classic x-ray findings are Codman triangle (periosteal elevation), “sun burst” pattern, and bone destruction. Treatment is surgery and chemotherapy. The prognosis is poor, but is improved with aggressive management such as resection of single pulmonary metastases (hematogenous metastasis to the lungs is common). **Secondary osteosarcoma** is seen in the elderly; these highly aggressive tumors are associated with Paget disease, irradiation, and chronic osteomyelitis.

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Figure 26-4. Osteosarcoma Seen in Fuzzy Calcifications Adjacent to Distal Femur

Grossly, osteosarcoma often involves the metaphyses of long bones, usually around the knee (distal femur and proximal tibia). It produces a large, firm, white-tan mass with necrosis and hemorrhage. Microscopically, anaplastic cells producing osteoid and bone are seen.

Chondrosarcoma is a malignant tumor of chondroblasts which may arise *de novo* or secondary to a preexisting enchondroma, exostosis, or Paget disease. Males are affected more frequently than females, with peak ages 30–60. It presents with enlarging mass with pain and swelling, and it typically involves the pelvic bones, spine, and shoulder girdle. Microscopically, there is cartilaginous matrix production. Radiographs show osteolytic destruction and “popcorn” calcification. The 5-year survival is predicted by histologic differentiation.

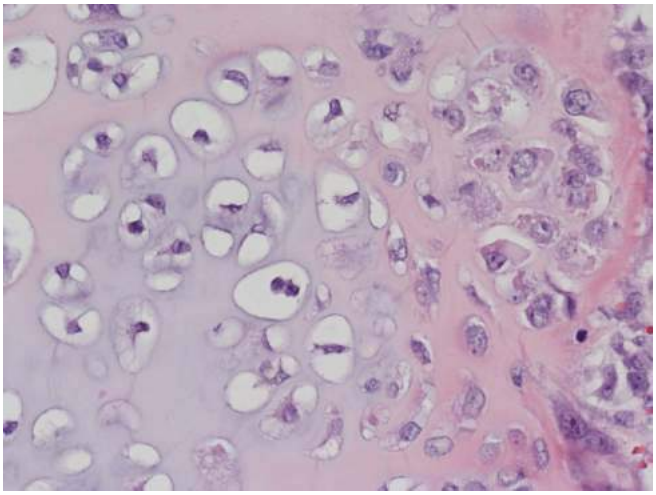

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Figure 26-5. Chondrosarcoma

Ewing sarcoma is a malignant neoplasm of undifferentiated cells arising within the marrow cavity. Males are affected slightly more often than females, with most cases in teenage years (ages 5–20). The classic translocation for Ewing sarcoma is t(11;22), which produces the EWS-FLI1 fusion protein.

Clinically, patients present with pain, swelling, and tenderness. X-ray studies show concentric "onion-skin" layering of new periosteal bone with soft tissue extension. Treatment is chemotherapy, surgery, and/or radiation; the 5-year survival rate is 75%.

Grossly, Ewing sarcoma often affects the diaphyses of long bones, with the most common sites being the femur, pelvis, and tibia. The tumor characteristically produces a white-tan mass with necrosis and hemorrhage. Microscopically, Ewing sarcoma is characterized by sheets of undifferentiated small, round, blue cells resembling lymphocytes, which may form Homer Wright pseudorosettes. The tumor cells erode through the cortex and periosteum and invade surrounding tissues.

Metastasis to bone is much more common than primary bone tumor. Common primary sites include prostate (often osteoblastic), breast, lung, thyroid, and kidney.

Joint Pathology 27

Learning Objectives

- ❑ Solve problems concerning osteoarthritis, rheumatoid arthritis, and seronegative spondyloarthropathies
- ❑ Describe arthritis related to crystal deposition, infectious arthritis, and neuropathic arthropathy (Charcot joint)

OSTEOARTHRITIS

Osteoarthritis (OA) (degenerative joint disease) is joint degeneration with loss of articular cartilage, with no to minimal inflammation. It is the most common form of arthritis. Risk increases with age; OA affects at least 1 joint in 80% of people age >70.

Clinically, there is an insidious onset of joint stiffness; deep, aching joint pain, which worsens with repetitive motion; decreased range of motion; crepitus; and joint effusions and swelling. Osteophytes may cause nerve compression. X-ray studies show narrowing of the joint space due to loss of cartilage; osteosclerosis and bone cysts; and osteophytes (osteophytic lipping).

The pathogenesis involves both biomechanical factors (aging or wear and tear of articular cartilage) and biochemical factors (chondrocyte injury and abnormal collagen activity). Predisposing factors include obesity, previous joint injury, ochronosis, diabetes, and hemarthrosis.

OA affects weight-bearing joints (knees, hips, and spine), often with asymmetrical involvement.

- There is degeneration and loss of articular cartilage with eburnation (exposed bone becomes polished) and subchondral bone sclerosis.
- The changes may include subchondral bone cysts, loose bodies (joint mice), which are free-floating fragments of cartilage and bone, and osteophytes (bone spurs), which are reactive bony outgrowths.
- **Heberden nodes** are osteophytes at the distal interphalangeal (DIP) joints, while **Bouchard nodes** are osteophytes at the proximal interphalangeal (PIP) joints.

Osteoarthritis vs RA

Osteoarthritis	RA
"Wear and tear"	Systemic autoimmune disease (+) Rheumatoid factor (+) Rheumatoid nodules
Degeneration of articular cartilage	Synovial proliferation
Knees and hands (DIP) in women; hips in men	Hands (PIP) and feet
Asymmetrical	Symmetrical and migratory

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Figure 27-1. Heberden (Distal Interphalangeal Joint) and Bouchard (Proximal Interphalangeal Joint) Nodes in Patient with Osteoarthritis

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a systemic, chronic, inflammatory disease characterized by progressive arthritis, production of rheumatoid factor, and extra-articular manifestations. It affects females 4x more than men, with highest incidence at ages 20–50. Some cases have a genetic predisposition (HLA-DR4 and -DR1).

RA is thought to be caused by an autoimmune reaction triggered by an infectious agent in a genetically susceptible individual.

RA most commonly affects the hand, wrist, knee, and ankle joints, and the involvement tends to be symmetrical. There is often morning stiffness which improves with activity.

- There is typically fusiform swelling, redness, and warmth of the proximal interphalangeal (PIP) joint.
- X-ray studies show juxta-articular osteoporosis and bone erosions; joint effusion may also be present.
- RA causes a diffuse proliferative synovitis, pannus formation (proliferation of the synovium and granulation tissue over the articular cartilage of the joint), fibrous and bony ankylosis (joint fusion), and joint deformities. Joint deformities can include:
  - Radial deviation of the wrist and ulnar deviation of the fingers
  - Swan neck deformity (hyperextension of PIP joint and flexion of DIP joint
  - Boutonniere deformity (flexion of PIP and extension of DIP joints)
- Baker cysts (synovial cysts in the popliteal fossa) may be present.

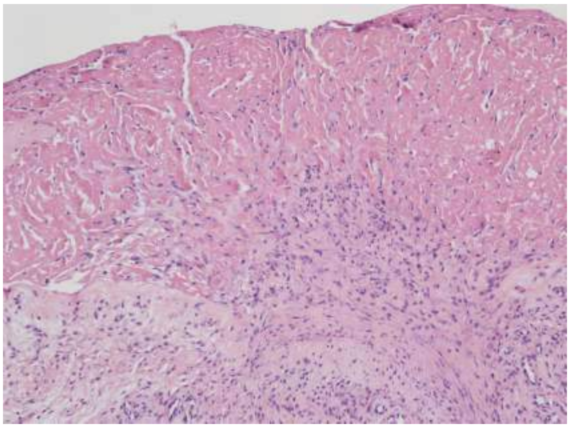

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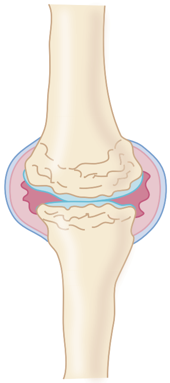
Figure 27-2. Pannus Formation (Rheumatoid Arthritis)

Lab studies show elevated sedimentation rate and hypergammaglobulinemia. Rheumatoid factor (RF) is an autoantibody (usually IgM) against the Fc fragment of IgG; it is present in 80% of cases. RF may circulate and form immune complexes, and titer of RF correlates with the severity of the arthritis and prognosis.

- Extra-articular manifestations may be prominent. Systemic symptoms include low-grade fever, malaise, fatigue, lymphadenopathy, and weakness. Arteries may show acute necrotizing vasculitis due to circulating antigen-antibody complexes.
- Rheumatoid nodules, subcutaneous skin nodules, are present in 25% of cases. They are usually found on extensor surfaces of the forearms and elbows, but can also be found in the heart valves, lung, pleura, pericardium, and spleen. They are composed of central fibrinoid necrosis surrounded by epithelioid macrophages, lymphocytes, and granulation tissue.
- **Sjögren syndrome** may be present in 15%. In **Felty syndrome**, RA accompanies splenomegaly and neutropenia. In **Caplan syndrome**, RA is associated with pneumoconiosis.
- Secondary amyloidosis may also complicate RA.



Rheumatoid Arthritis

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Figure 27-3. Ulnar Deviation at the Metacarpophalangeal Joints in Advanced Rheumatoid Arthritis

SERONEGATIVE SPONDYLOARTHROPATHIES

Seronegative spondyloarthropathies are a group of disorders characterized by the following:

- Rheumatoid factor seronegativity
- Involvement of the sacroiliac joints
- Association with HLA-B27

Ankylosing spondylitis occurs predominantly in young men with HLA-B27 (90% of cases); usually involves the sacroiliac joints and spine; and may be associated with inflammatory bowel disease.

Clinical Correlate

Complete fusion of the spine can occur in ankylosing spondylitis and can cause complete rigidity of the spine. The resulting condition is known as **bamboo spine**, which can be seen on x-ray.

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Figure 27-4. Ankylosing Spondylitis Can Cause Fusion of Vertebrae, Leading to a "Bamboo Spine"

Reactive arthritis is characterized by a classic triad of conjunctivitis, urethritis, and arthritis. The arthritis affects the ankles and knees. It affects males more than females, with onset age 20s–30s. Onset often follows a venereal disease or bacillary dysentery.

Enteropathic arthritis occurs in 10–20% of patients with inflammatory bowel disease.

Psoriatic arthritis affects 5–10% of patients with psoriasis; is often a mild and slowly progressive arthritis, with pathology similar to RA.

ARTHRITIS RELATED TO CRYSTAL DEPOSITION

In **gout**, hyperuricemia and the deposition of monosodium urate crystals in joints will result in recurrent bouts of acute arthritis. The hyperuricemia can be caused by overproduction or underexcretion of uric acid.

- **Primary gout** (90%) is idiopathic, affects males more than females, and is typically seen in older men.
- **Secondary gout** (10%) is seen with excessive cell breakdown (chemotherapy), decreased renal excretion (drugs), and Lesch-Nyhan syndrome.

Gout affects the great toe (podagra, characterized by an exquisitely painful, inflamed big toe), ankle, heel, and wrist.

Joint aspiration shows birefringent, needle-shaped uric acid crystals and numerous neutrophils. **Tophi** are deposits of crystals surrounded by inflammation. Skin ulceration and destruction of adjacent joints may occur. Complications include joint destruction and deformity, uric acid renal calculi, and renal failure. Treatment is NSAIDs, colchicine, probenecid, and allopurinol.

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Figure 27-5. Gout

Pseudogout (chondrocalcinosis) is deposition of calcium pyrophosphate crystals in joints, leading to inflammation. Affected patients are usually age >50. The knee joint is most commonly involved. Aspiration of the joint demonstrates positively birefringent (weak), rhomboid-shaped crystals. Pseudogout is associated with many metabolic diseases (e.g., diabetes, hypothyroidism, ochronosis), and it may mimic OA or RA.

INFECTIOUS ARTHRITIS

Suppurative arthritis may result from seeding of the joint during bacteremia. Other routes include spread from an adjacent site of infection and direct inoculation. Infecting organisms include gonococci, *Staphylococcus, Streptococcus*, *H. influenzae*, and gram-negative bacilli.

Suppurative arthritis causes a tender, painful, swollen, and erythematous joint. Large joints (knee, hip, shoulder) are most often infected, and the arthritis is usually monoarticular. Joint aspiration shows cloudy synovial fluid that clots

Bridge to Biochemistry

Uric acid is the end product of purine metabolism.

Note

Lesch-Nyhan Syndrome

- X-linked recessive
- Deficiency of hypoxanthine-guanine phosphoribosyl-transferase (HPRT)
- Cognitive impairment
- Dystonia
- Self-mutilating behaviors
- Hyperuricemia

Note

In the presence of pseudogout age <50, suspect one of these metabolic abnormalities (4H):

- Hemachromatosis
- Hyperparathyroidism
- Hypophosphatemia
- Hypomagnesemia

readily and has a high neutrophil count. Gram stain and culture are positive in 50–70% of cases. Treatment is rapid intervention with antibiotics to prevent permanent joint damage.

Lyme disease is caused by the spirochete *Borrelia burgdorferi*. The disease is arthropod-borne, spread by deer ticks (*Ixodes dammini*). Symptoms are skin rash (erythema chronicum migrans), and migratory arthritis involving the knees, shoulders, and elbows. The histology of the arthritic joint is similar to RA. Lyme disease can also have CNS and cardiac involvement. Serologic tests may remain negative until infection has been present for several weeks.

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Figure 27-6. Large Targetoid Lesion of Erythema Chronicum Migrans Considered Pathognomonic of Lyme Disease

NEUROPATHIC ARTHROPATHY (CHARCOT JOINT)

Charcot joint refers to joint damage secondary to impaired joint innervation (neuropathy), leading to an inability to sense pain. The damage also leads to destruction of joint surfaces, debris in joints, deformity, and dislocations.

Different **underlying neurologic diseases** tend to affect different joints.

- Diabetes mellitus (most common cause) tends to damage the tarsometatarsal joint in the midfoot.
- Syringomyelia (cavity in spinal cord) tends to damage the shoulder, elbow, and wrist joints.
- Tabes dorsalis (neurosyphilis) tends to damage the hip, knee, and ankle joints.

Bridge to Microbiology

Arthropod-borne diseases transmitted by ticks include:

- Rocky Mountain spotted fever
- *Ehrlichia*
- Babesiosis
- Tularemia
- Lyme disease

Skeletal Muscle and Peripheral Nerve Pathology 28

Learning Objectives

- ❑ Demonstrate understanding of inflammatory myopathies and neuropathies
- ❑ Describe the mechanism of action of myasthenic syndromes
- ❑ Explain information related to muscular dystrophy
- ❑ Solve problems concerning soft tissue and peripheral nerve tumors

Type I (red) skeletal muscle is used in postural weight bearing. It produces a slow twitch as a result of aerobic metabolism of fatty acids.

Type II (white) skeletal muscle is used for purposeful movement. It produces a fast twitch as a result of anaerobic glycolysis of glycogen.

Note

Skeletal muscle fiber type is determined by innervation.

Table 28-1. Type I (Slow-Twitch) Versus Type II (Fast-Twitch) Muscles

	Type I	Type II
Twitch rate	Slow twitch	Fast twitch
Function	Postural weight bearing Sustained tension	Purposeful movement Short, quick bursts
Metabolism	Aerobic (Krebs cycle)	Anaerobic (glycolysis)
Energy source	Fatty acids	Glycogen
Mitochondria	Many	Few
Color	Red	White
Fatigue	Slow fatigue	Rapid fatigue

INFLAMMATORY MYOPATHIES

Polymyositis is an autoimmune disease seen in adults. It presents with bilateral proximal muscle weakness. Microscopic exam demonstrates endomysial lymphocytic inflammation (mostly cytotoxic T8) and skeletal muscle fiber degeneration and regeneration. Patients respond to immunosuppression.

Clinical Correlate

Anti-tRNA synthetase antibodies such as the anti-Jo-1 antibody are known to be highly specific for inflammatory myopathies.

Dermatomyositis is a connective tissue disorder involving inflammation of skeletal muscle and skin. It can affect both children and adults. It presents with bilateral proximal muscle weakness, skin rash of the upper eyelids, and periorbital edema.

Microscopic exam demonstrates perimysial and vascular lymphocytic inflammation, perifascicular fiber atrophy, and skeletal muscle fiber degeneration and regeneration. Adult patients are at increased risk of lung, colon, breast, and gynecologic cancers.

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Figure 28-1. Periorbital Heliotrope Rash of Dermatomyositis

Inclusion body myositis affects adults age >50, causing slowly progressive, asymmetrical, distal muscle weakness. Light microscopy demonstrates autophagic vacuoles and inclusion bodies in addition to inflammation and necrosis. The disease is refractory to immunosuppressive therapy.

MYASTHENIC SYNDROMES

Myasthenia gravis is an autoimmune disease characterized by autoantibodies against the acetylcholine (ACh) receptor of the neuromuscular junction, resulting in muscular weakness predominantly affecting the facial muscles. Females are affected more frequently than males.

- Extraocular muscle weakness may lead to ptosis and diplopia; the weakness worsens with repeated contractions.
- Respiratory muscle involvement may lead to death.
- There is an association with thymic hyperplasia and thymomas.

Treatment is anticholinesterase agents, steroids, and thymectomy.

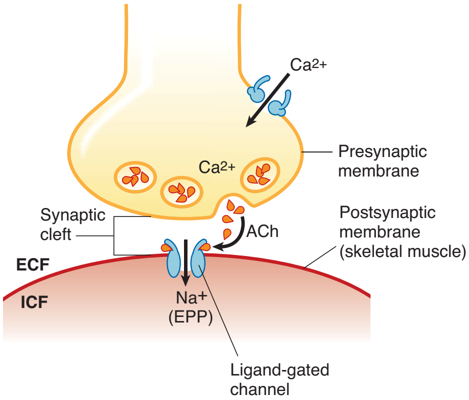

Figure 28-2. Neuromuscular Transmission

Lambert-Eaton myasthenic syndrome frequently arises before a diagnosis of cancer is made, often in cases of small cell lung cancer. Patients report dry mouth and proximal muscle weakness. Autoantibodies are directed against presynaptic calcium channels of the neuromuscular junction. Treatment is immunotherapy and cancer treatment, if indicated.

MUSCULAR DYSTROPHY

Duchenne muscular dystrophy is a recessive X-linked form of muscular dystrophy leading to rapid progression of muscle degeneration. It is the most common and severe form of muscular dystrophy. The affected gene is the dystrophin gene on the X chromosome (Xp21); dystrophin protein is an important muscle structural protein, and mutation results in a virtual absence of the dystrophin protein.

Affected boys are normal at birth but have onset of symptoms by age 5. Clinical features include:

- Progressive muscular weakness
- Calf pseudohypertrophy
- Proximal weakness of shoulder and pelvic girdles
- Possible heart failure and arrhythmias
- Respiratory insufficiency and pulmonary infections as a result of decreased mucociliary clearance

Lab studies show elevated serum creatine kinase. Muscle biopsy shows muscle fibers of various sizes; necrosis, degeneration, and regeneration of fibers; fibrosis; and fatty infiltration. Immunostains show decreased dystrophin protein. Diagnosis can also be confirmed with genetic testing.

Becker muscular dystrophy is a recessive X-linked inherited disorder leading to slowly progressive muscle weakness of the legs and pelvis.

- Less severe than Duchenne
- Not as common as Duchenne
- Has a later onset than Duchenne, with variable progression
- Mutation produces an altered dystrophin protein
- Cardiac involvement is rare, and patients can have relatively normal lifespan

Note the characteristic pattern of rising from the floor using a sequence of hand pushes

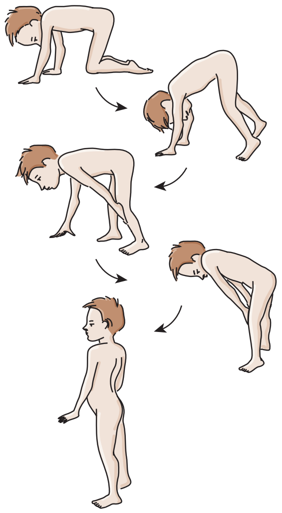

Figure 28-3. Gower Sign in Duchenne Muscular Dystrophy

INFLAMMATORY NEUROPATHY

Guillain-Barré syndrome is an autoimmune disease leading to the destruction of Schwann cells and peripheral nerve demyelination. Clinically, it is preceded by a viral illness. Muscular weakness occurs with an ascending paralysis, accompanied by loss of deep tendon reflexes. Diagnosis can be established with nerve conduction studies; lumbar puncture shows elevated protein.

Microscopic examination demonstrates inflammation and demyelination of peripheral nerves and spinal nerve roots, resulting in muscular weakness. Guillain-Barré syndrome is fatal in 5% of cases because of respiratory paralysis. Treatment is plasmapheresis and immunoglobulin therapy.

SOFT TISSUE AND PERIPHERAL NERVE TUMORS

Lipoma is a benign adipose tissue tumor that most often arises in subcutaneous tissue of trunk, neck, or proximal extremities. It is the most common benign soft tissue tumor. The tumor is usually more of a cosmetic problem than a medical one. Microscopically, it is composed of mature fat cells but can contain other mesenchymal elements.

Liposarcoma is a malignant adipose tissue tumor that most often arises in the thigh or retroperitoneum. It is the most common adult sarcoma. It is distinguished from lipoma by the presence of **lipoblasts**. Grossly, it tends to be larger than lipoma, and the cut surface shows fibrous bands. Microscopically, well-differentiated liposarcoma consists of mature fat with varying numbers of hyperchromatic spindle cells and multivacuolated lipoblasts. Metastases are rare but retroperitoneal tumors tend to recur.

Dermatofibroma is a benign dermal spindle cell proliferation that most often arises in the extremities. A small, red nodule is seen, which is tender and mobile on examination.

Fibromatosis is a non-neoplastic proliferative connective tissue disorder that can histologically resemble a sarcoma. Fibrous tissue infiltrates muscle or other

tissue, and may cause a mass lesion. The cut surface is trabeculated. Microscopically, bundles of fibroblasts and collagen are seen.

- **Superficial fibromatoses** arise from fascia or aponeuroses. Palmar fibromatosis is the most common type. Penile fibromatosis is known as Peyronie's disease.
- **Deep fibromatoses** (desmoids) occur in extraabdominal sites (children) and abdominal wall and extraabdominal sites (adults). Abdominal desmoids often occur in women within a year of pregnancy. They may also follow surgery or trauma. Intraabdominal fibromatosis is commonly associated with Gardner syndrome (*see* Gastrointestinal Tract Pathology, chapter 16).

Fibrosarcoma is a malignant fibrous tumor, commonly seen on the thigh and upper limb. It may arise spontaneously or after therapeutic/accidental irradiation. Microscopically, there are uniform spindle cells with a "herringbone" pattern. Metastases are hematogenous, often to the lung.

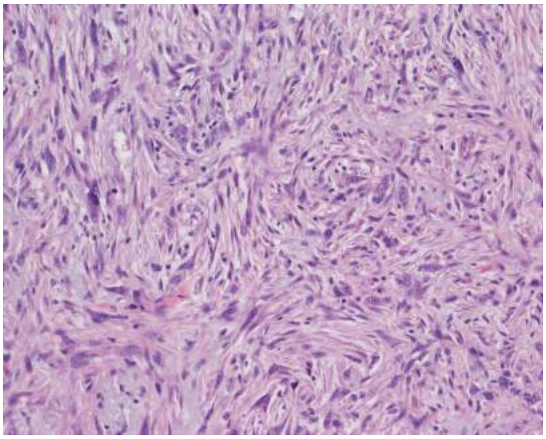

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Figure 28-4. Fibrosarcoma

Undifferentiated pleomorphic sarcoma (previously known as malignant fibrous histiocytoma) is a large multilobulated tumor seen in the extremities and retroperitoneum of older adults. Microscopically, they may have a storiform (cartwheel-like) pattern. They recur and metastasize.

Rhabdomyoma (*See* Female Genital and Cardiac Pathology, chapters 22 and 13.)

Embryonal rhabdomyosarcoma (*See* Female Genital chapter.)

Leiomyoma is a benign smooth muscle tumor most often seen in the uterus (*see* Female Genital chapter) and gastrointestinal tract. Less often it is seen in skin, and only rarely in deep soft tissue.

Leiomyosarcoma of soft tissue is less common than its counterpart in the gastrointestinal tract and uterus (*see* Female Genital chapter). In soft tissue, it usually arises in the retroperitoneum of older women.

Grossly, the tumor is fleshy and white with hemorrhage and necrosis. Microscopically, the tumor nuclei are blunt ended ("cigar-shaped"). Longitudinal

striations can be seen with Masson trichrome staining. The tumor is highly aggressive in the retroperitoneum, where complete resection may not be possible.

Synovial sarcoma occurs in young adults. The knee is a common location. The gross appearance is variable but calcification is common. Microscopically, tumors may be biphasic (epithelial and spindle cells) or monophasic (spindle cell or epithelial).

Benign peripheral nerve sheath tumors

- **Schwannoma** is an encapsulated nerve sheath tumor with alternating Antoni A and B areas (*see* Central Nervous System, chapter 20). There is an association with NF2.
- **Neurofibroma** is nonencapsulated and may have a solitary, diffuse, or plexiform pattern. Microscopically, neoplastic cells are interspersed among wavy, loose or dense collagen bundles. There is an association with NF1 (*see* Genetic Disorders, chapter 6).

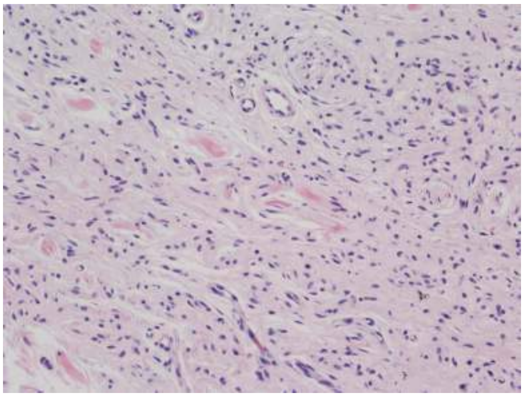

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Figure 28-5. Neurofibroma

Malignant peripheral nerve sheath tumor may arise from neurofibromas or *de novo* in a peripheral nerve. It typically occurs in young adults in major nerve trunks (sciatic nerve, brachial plexus, and sacral plexus). Microscopically, it resembles fibrosarcoma. Recurrence and distant metastases are common.

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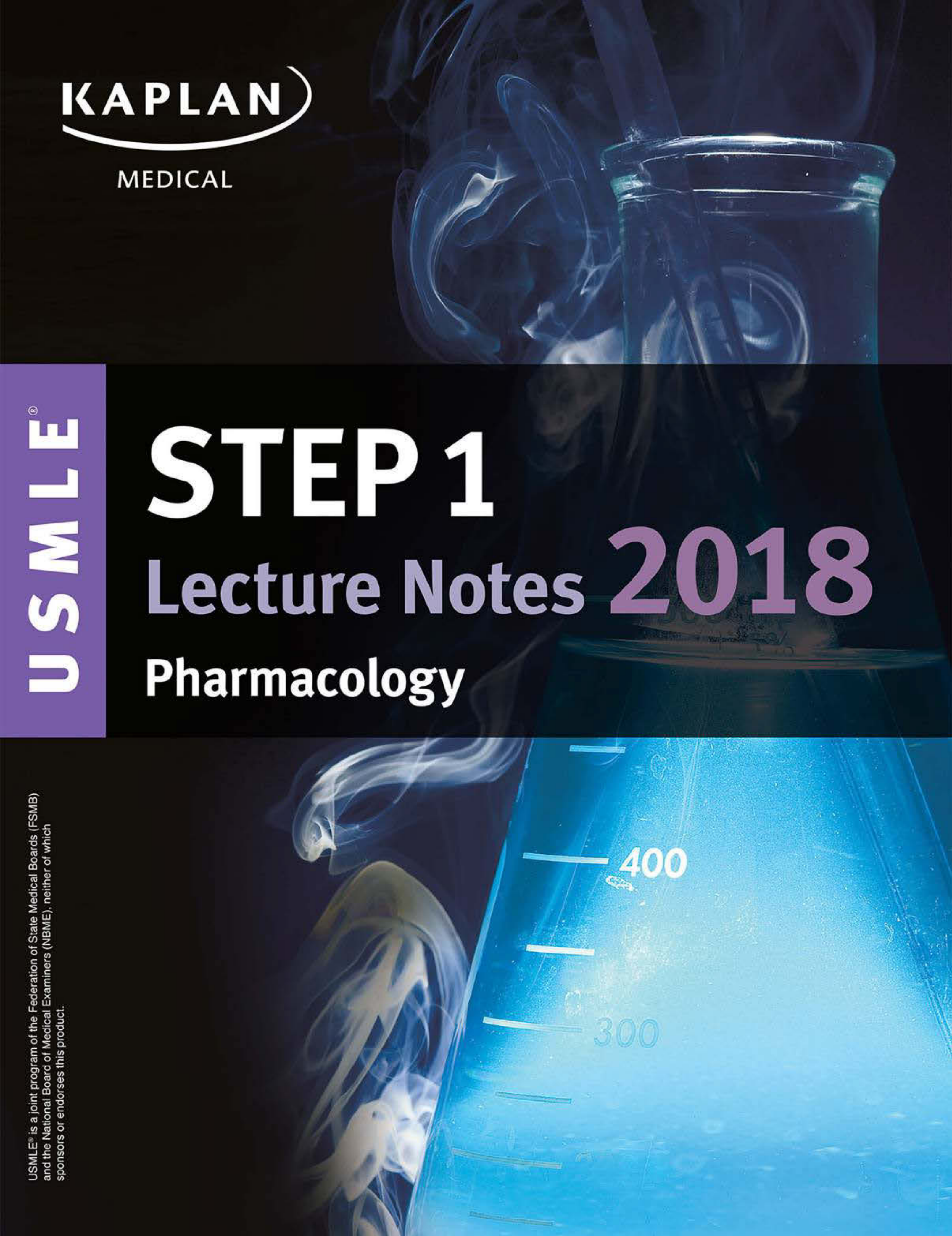

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PART I

General Principles

Pharmacokinetics 1

Learning Objectives

- ❑ Answer questions about permeation, absorption, distribution, biotransformation, elimination, and steady state
- ❑ Solve problems concerning important pharmacokinetics calculations

PHARMACOKINETICS

Pharmacokinetic characteristics of drug molecules concern the processes of absorption, distribution, metabolism, and excretion. The biodisposition of a drug involves its permeation across cellular membrane barriers.

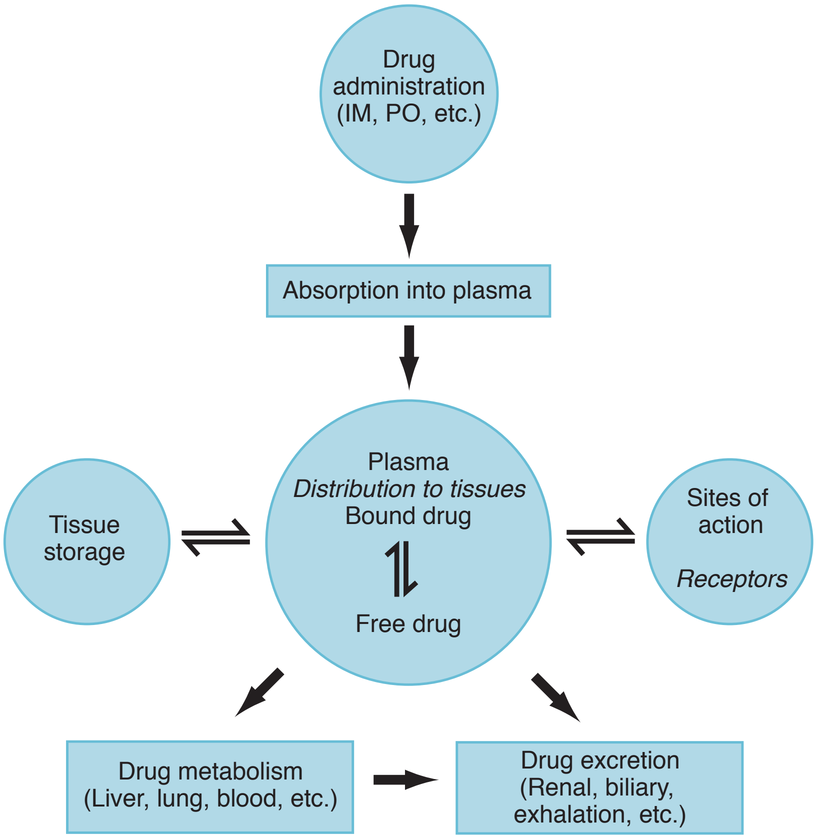


Figure I-1-1. Drug Biodisposition

Permeation

Drug permeation is dependent on the following:

- **Solubility.** Ability to diffuse through lipid bilayers (lipid solubility) is important for most drugs; however, water solubility can influence permeation through aqueous phases.
- **Concentration gradient.** Diffusion down a concentration gradient—only free, unionized drug forms contribute to the concentration gradient.
- **Surface area and vascularity.** Important with regard to absorption of drugs into the systemic circulation. The larger the surface area and the greater the vascularity, the better is the absorption of the drug.

Ionization

High-Yield

Many drugs are weak acids or weak bases, and can exist in either nonionized or ionized forms in an equilibrium, depending on the pH of the environment and the pKa (the pH at which the molecule is 50% ionized and 50% nonionized).

- Only the nonionized (uncharged) form of a drug crosses biomembranes.
- The ionized form is better renally excreted because it is water soluble.

Note

For Weak Acids and Weak Bases

Ionized = water soluble

Nonionized = lipid soluble

Weak Acid	R–COOH (crosses membranes)	$\rightleftharpoons$	$R–COO^- + H^+$ (better cleared)
Weak Base	$R–NH_3^+$ (better cleared)	$\rightleftharpoons$	$R–NH_2 + H^+$ (crosses membranes)

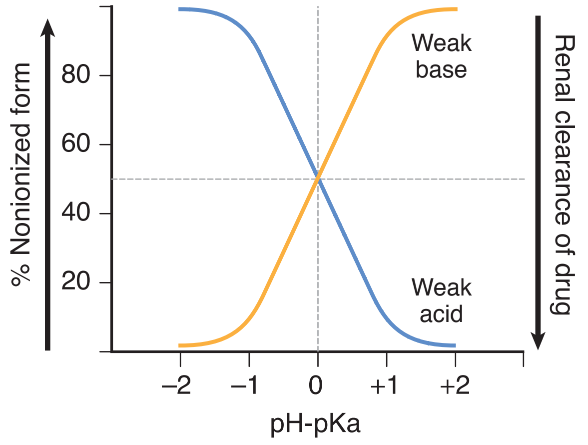


Figure I-1-2. Degree of Ionization and Clearance Versus pH Deviation from pKa

Clinical Correlate

Gut bacteria metabolize lactulose to lactic acid, acidifying the fecal masses and causing ammonia to become ammonium. Therefore, lactulose is useful in hepatic encephalopathy.

Ionization Increases Renal Clearance of Drugs

High-Yield

Only free, unbound drug is filtered. Both ionized and nonionized forms of a drug are filtered.

- Only nonionized forms undergo active secretion and active or passive reabsorption.
- Ionized forms of drugs are "trapped" in the filtrate.
- Acidification of urine → increases ionization of weak bases → increases renal elimination.
- Alkalinization of urine → increases ionization of weak acids → increases renal elimination.

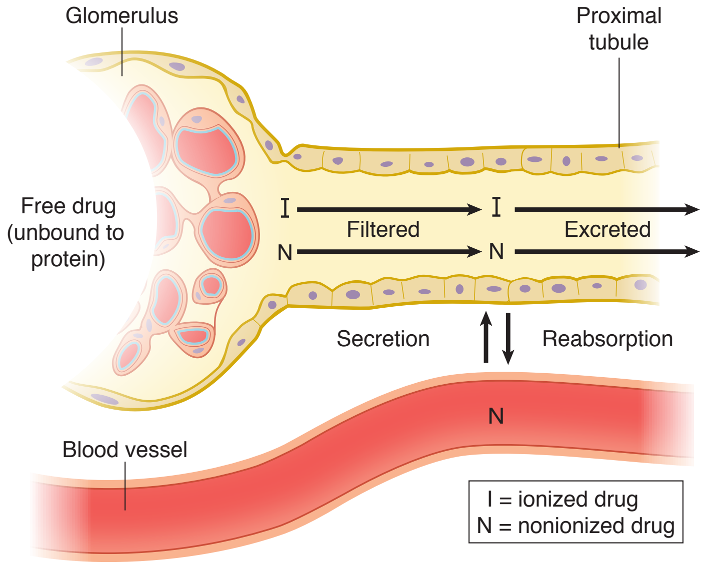


Figure I-1-3. Renal Clearance of Drug

Modes of Drug Transport Across a Membrane

Table I-1-1. The 3 Basic Modes of Drug Transport Across a Membrane

Mechanism	Direction	Energy Required	Carrier	Saturable
Passive diffusion	Down gradient	No	No	No
Facilitated diffusion	Down gradient	No	Yes	Yes
Active transport	Against gradient (concentration/ electrical)	Yes	Yes	Yes

Clinical Correlate

To Change Urinary pH

- Acidify: NH_4Cl, vitamin C, cranberry juice
- Alkalinize: $NaHCO_3$, acetazolamide (historically)
- *See* Aspirin Overdose and Management in Section VI.

Bridge to Physiology

Ion and molecular transport mechanisms are discussed in greater detail in Part I of Physiology.

ABSORPTION

Absorption concerns the processes of entry of a drug into the systemic circulation from the site of its administration. The determinants of absorption are those described for drug permeation.

- Intravascular administration (e.g., IV) does not involve absorption, and there is no loss of drug. Bioavailability = 100%
- With extravascular administration (e.g., per os [PO; oral], intramuscular [IM], subcutaneous [SC], inhalation), less than 100% of a dose may reach the systemic circulation because of variations in bioavailability.

Plasma Level Curves

C_{max} = maximal drug level obtained with the dose.

t_{max} = time at which C_{max} occurs.

Lag time = time from administration to appearance in blood.

Onset of activity = time from administration to blood level reaching minimal effective concentration (MEC).

Duration of action = time plasma concentration remains greater than MEC.

Time to peak = time from administration to C_{max}.

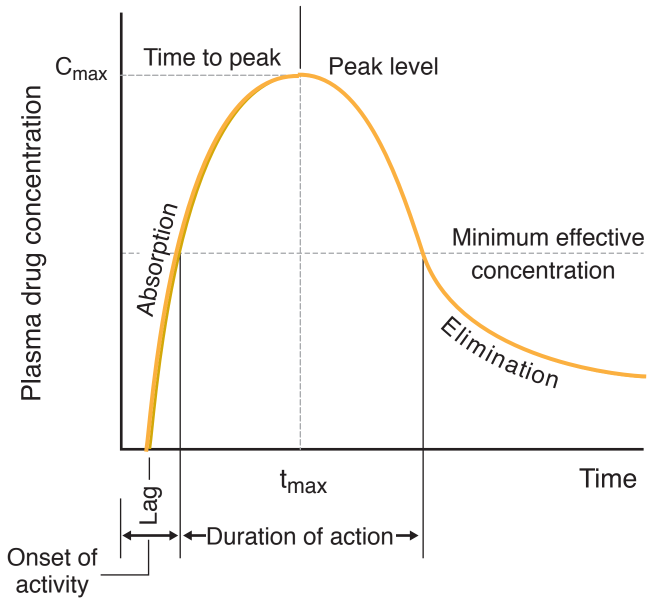


Figure I-1-4. Plot of Plasma Concentration Versus Time

Bioavailability (f)

Bioavailability is the measure of the fraction of a dose that reaches the systemic circulation. By definition, intravascular doses have 100% bioavailability, f = 1.

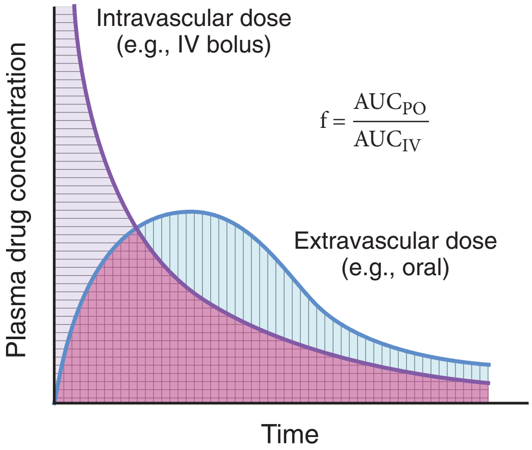


Figure I-1-5. Area Under the Curve for an IV Bolus and Extravascular Doses

AUC: area under the curve

PO: oral

IV: intravenous bolus

AUC_{IV}: horizontally striped area

AUC_{PO}: vertically striped area

First-Pass Effect

With oral administration, drugs are absorbed into the portal circulation and initially distributed to the liver. For some drugs, their rapid hepatic metabolism decreases bioavailability, i.e., the "first-pass" effect. Examples include lidocaine (IV vs. PO) and nitroglycerin (sublingual).

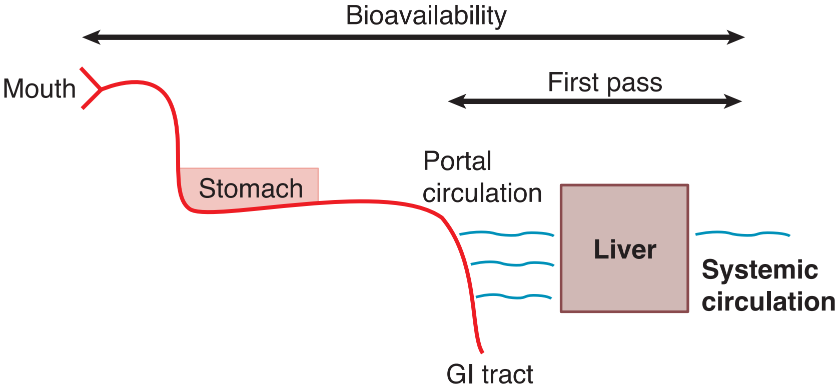


Figure I-1-6. Bioavailability and First-Pass Metabolism

Clinical Correlate

Drugs with high plasma protein binding and narrow therapeutic range (e.g., warfarin, phenytoin) are prone to drug interactions.

DISTRIBUTION

Distribution is the process of distribution of a drug from the systemic circulation to organs and tissue.

- Under normal conditions, protein-binding capacity is much larger than is drug concentration. Consequently, the free fraction is generally constant.
- Many drugs bind to plasma proteins, including albumin, with an equilibrium between bound and free molecules (recall that only unbound drugs cross biomembranes).

$$\underset{\text{(Active, free)}}{\text{Drug} + \text{Protein}} \rightleftharpoons \underset{\text{(Inactive, bound)}}{\text{Drug-Protein Complex}}$$

- Competition between drugs for plasma protein-binding sites may increase the "free fraction," possibly enhancing the effects of the drug displaced. Example: sulfonamides and bilirubin in a neonate

Special Barriers to Distribution

There are some special barriers to distribution:

- Placental: most small molecular weight drugs cross the placental barrier, although fetal blood levels are usually lower than maternal (e.g., propylthiouracil [PTU] versus methimazole in pregnancy)
- Blood–brain: permeable only to lipid-soluble drugs or those which are transported by facilitated diffusion or active transport." (e.g., levodopa versus dopamine)

Bridge to Physiology

Approximate V_d Values (weight 70 kg)

- Plasma volume (3 L)
- Blood volume (5 L)
- Extracellular fluid (ECF 12–14 L)
- Total body water (TBW 40–42 L)

Apparent Volume of Distribution

High-Yield

Apparent volume of distribution (V_d) is a kinetic parameter of a drug which correlates dose with plasma level at zero time.

$$V_d = \frac{\text{Dose}}{C^0} \quad \text{where} \quad C^0 = [\text{plasma}] \text{ at zero time}$$

This relationship can be used for calculating V_d by using the **dose** only if one knows C^0.

- V_d is low when a high percentage of a drug is bound to plasma proteins.
- V_d is high when a high percentage of a drug is being sequestered in tissues. This raises the possibility of displacement by other agents; examples: verapamil and quinidine can displace digoxin from tissue-binding sites.
- V_d is needed to calculate a loading dose in the clinical setting (*see* Pharmacokinetic Calculation section, Equation 4).

Redistribution

In addition to crossing the blood–brain barrier (BBB), lipid-soluble drugs redistribute into fat tissues prior to elimination.

In the case of CNS drugs, the duration of action of an initial dose may depend more on the redistribution rate than on the half-life. With a second dose, the blood/fat ratio is less; therefore, the rate of redistribution is less and the second dose has a longer duration of action.

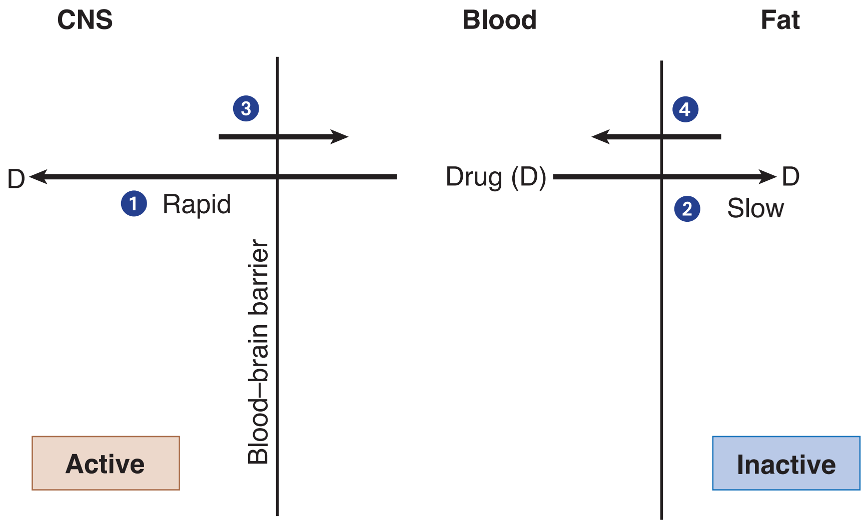


Figure I-1-7. Redistribution

BIOTRANSFORMATION

The general principle of biotransformation is the metabolic conversion of drug molecules to more water-soluble metabolites that are more readily excreted.

- In many cases, metabolism of a drug results in its conversion to compounds that have little or no pharmacologic activity.
- In other cases, biotransformation of an active compound may lead to the formation of metabolites that also have pharmacologic actions.
- A few compounds (**prodrugs**) have no activity until they undergo metabolic activation.
- Some compounds are converted to toxic metabolites, e.g., acetaminophen.

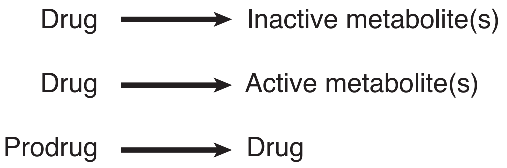


Figure I-1-8. Biotransformation of Drugs

Clinical Correlate

Active Metabolites

Biotransformation of the benzodiazepine diazepam results in formation of nordiazepam, a metabolite with sedative-hypnotic activity and a long duration of action.

Biotransformation Classification

High-Yield

There are two broad types of biotransformation, phase I and phase II.

Phase I

Phase I biotransformation is modification of the drug molecule via oxidation, reduction, or hydrolysis.

- **Microsomal metabolism**
  - **Cytochrome P450 isozymes**: major enzyme systems involved in phase I reactions; localized in smooth endoplasmic reticulum (microsomal fraction) of cells (especially liver but also GI tract, lungs, kidney)
  - P450s have an absolute requirement for molecular oxygen and NADPH
  - Oxidations include hydroxylations and dealkylations
  - Multiple CYP families differing by amino acid (AA) composition, by substrate specificity, and by sensitivity to inhibitors and to inducing agents

Clinical Correlate

Active components in **grapefruit juice** include furanocoumarins capable of inhibiting the metabolism of many drugs, including alprazolam, midazolam, atorvastatin, and cyclosporine. Such compounds may also enhance oral bioavailability decreasing first-pass metabolism and by inhibiting drug transporters in the GI tract responsible for intestinal efflux of drugs.

Table I-1-2. Cytochrome P450 Isozymes

CYP450	Substrate Example	Inducers	Inhibitors	Genetic Polymorphisms
1A2	Theophylline Acetaminophen	Aromatic hydrocarbons (smoke) Cruciferous vegetables	Quinolones Macrolides	No
2C9	Phenytoin Warfarin	General inducers*	—	Yes
2D6	Many cardiovascular and CNS drugs	None known	Haloperidol Quinidine SSRIs	Yes
3A4	60% of drugs in PDR	General inducers*	General inhibitors† Grapefruit juice	No

* General inducers: anticonvulsants (barbiturates, phenytoin, carbamazepine), antibiotics (rifampin), chronic alcohol, St. John's Wort.

† General inhibitors: antiulcer medications (cimetidine, omeprazole), antimicrobials (chloramphenicol, macrolides, ritonavir, ketoconazole), acute alcohol.

- **Nonmicrosomal metabolism**
  - **Hydrolysis**: phase I reaction involving addition of a water molecule with subsequent bond breakage; includes esterases and amidases
  - Genetic polymorphism exists with pseudocholinesterases; examples include local anesthetics and succinylcholine
  - **Monoamine oxidases**: metabolism of endogenous amine neurotransmitters (dopamine, norepinephrine, and serotonin); metabolism of exogenous compounds (tyramine)
  - **Alcohol metabolism**: alcohols are metabolized to aldehydes and then to acids by dehydrogenases (*see* CNS Pharmacology, part IV); genetic polymorphisms exist

Phase II

Phase II biotransformation is conjugation with endogenous compounds via the activity of transferases. It may follow phase I or occur directly. Types of conjugation include:

- **Glucuronidation**
  - Inducible; may undergo enterohepatic cycling (drug: glucuronide → intestinal bacterial glucuronidases → free drug)
  - Reduced activity in neonates, chloramphenicol and gray baby syndrome
  - Morphine is activated
- **Acetylation**
  - Genotypic variations (fast and slow metabolizers)
  - Drug-induced SLE by slow acetylators with hydralazine > procainamide > isoniazid (INH)
- **Glutathione (GSH) conjugation**
  - Depletion of GSH in liver is associated with acetaminophen hepatotoxicity

Recall Question

Which of the following routes of administration has the highest bioavailability?

A. Intramuscular

B. Intravascular

C. Oral

D. Subcutaneous

E. Sublingual

Answer: B

Clinical Correlate

Elimination of a drug from the body does not always end the therapeutic effect. Irreversible inhibitors, e.g. aspirin, PPIs, MAOIs, have a therapeutic effect long after the drug is eliminated.

ELIMINATION

Elimination concerns the processes involved in the elimination of drugs from the body (and/or plasma) and their kinetic characteristics. The major modes of drug elimination are:

- Biotransformation to inactive metabolites
- Excretion via the kidney
- Excretion via other modes, including the bile duct, lungs, and sweat

The time to eliminate 50% of a given amount (or to decrease plasma level to 50% of a former level) is called the **elimination half-life** ($t_{1/2}$).

Zero-Order Elimination Rate

With zero-order elimination rate, a constant amount of drug is eliminated per unit time. If 80 mg is administered and 10 mg is eliminated every 4 h, the time course of drug elimination is:

$$80 \text{ mg} \xrightarrow{4\text{ h}} 70 \text{ mg} \xrightarrow{4\text{ h}} 60 \text{ mg} \xrightarrow{4\text{ h}} 50 \text{ mg} \xrightarrow{4\text{ h}} 40 \text{ mg}$$

The rate of elimination is independent of plasma concentration (or amount in the body).

- Drugs with zero-order elimination have no fixed half-life ($t_{1/2}$ is a variable)
- Drugs with zero-order elimination include ethanol (except low blood levels), phenytoin (high therapeutic doses), and salicylates (toxic doses)

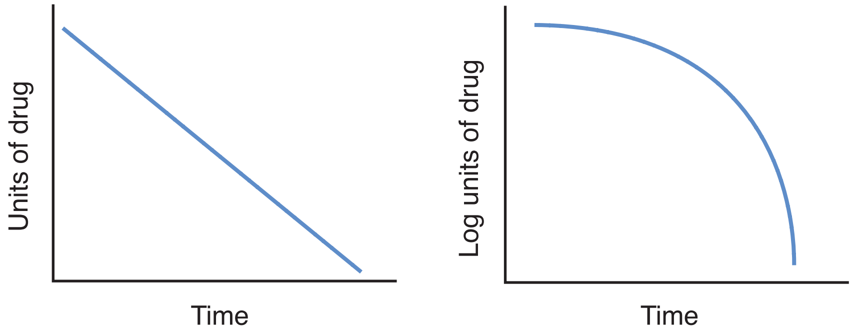


Figure I-1-9a. Plots of Zero-Order Kinetics

First-Order Elimination Rate

With first-order elimination rate, a constant fraction of the drug is eliminated per unit time ($t_{1/2}$ is a constant). Graphically, first-order elimination follows an exponential decay versus time. If 80 mg of a drug is administered and its elimination half-life = 4 h, the time course of its elimination is:

$$80 \text{ mg} \xrightarrow{4\text{ h}} 40 \text{ mg} \xrightarrow{4\text{ h}} 20 \text{ mg} \xrightarrow{4\text{ h}} 10 \text{ mg} \xrightarrow{4\text{ h}} 5 \text{ mg}$$

The rate of elimination is directly proportional to plasma level (or the amount present), i.e., the higher the amount, the more rapid the elimination.

- Most drugs follow first-order elimination rates
- $t_{1/2}$ is a constant

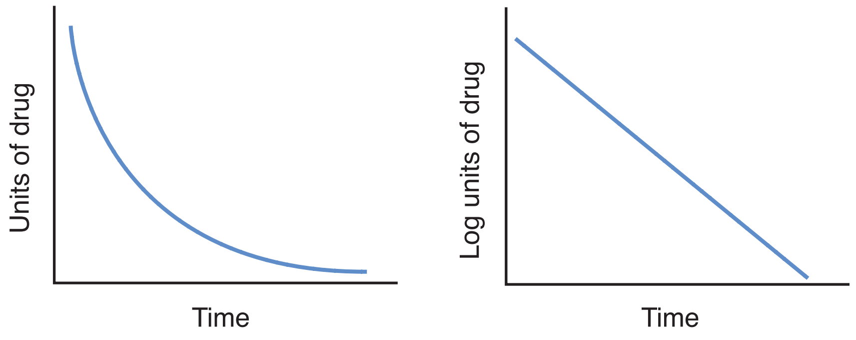


Figure I-1-9b. Plots of First-Order Kinetics

Graphic Analysis

Example of a graphic analysis of $t_{1/2}$:

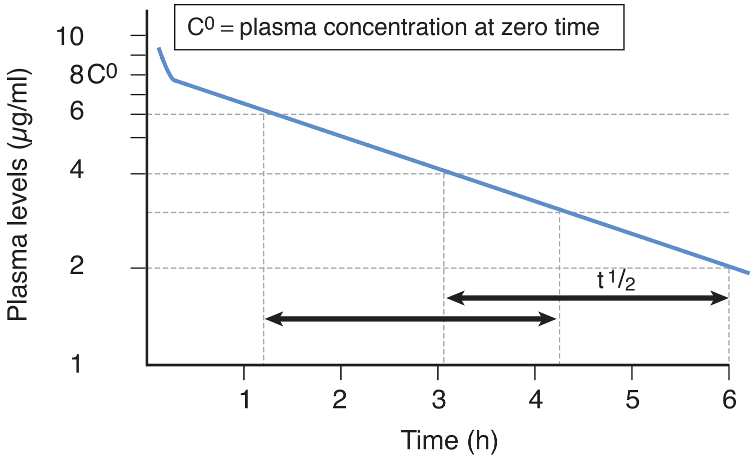


Figure I-1-10. Plasma Decay Curve—First-Order Elimination

The figure shows a plasma decay curve of a drug with first-order elimination plotted on semilog graph paper. The elimination half-life ($t_{1/2}$) and theoretical plasma concentration at zero time (C^0) can be estimated from the graphic relationship between plasma concentrations and time. C^0 is estimated by extrapolation of the linear plasma decay curve to intercept with the vertical axis.

Note

Elimination Kinetics

- Most drugs follow first order: rate falls as plasma level falls.
- Zero order is due to saturation of elimination mechanisms; e.g., drug-metabolizing reactions have reached Vmax.
- Zero order-elimination rate is constant; $t_{1/2}$ is a variable.
- First order-elimination rate is variable; $t_{1/2}$ is a constant.

Bridge to Renal Physiology

Inulin clearance is used to estimate GFR because it is not reabsorbed or secreted. A normal GFR is close to 120 mL/min.

Renal Elimination

The rate of elimination is the glomerular filtration rate (GFR) + active secretion – reabsorption (active or passive).

- Filtration is a nonsaturable linear function. Ionized and nonionized forms of drugs are filtered, but protein-bound drug molecules are not.
- Clearance (Cl) is the volume of blood cleared of drug per unit of time
  - Cl is constant in first-order kinetics
  - Cl = GFR when there is no reabsorption or secretion and no plasma protein binding
  - Protein-bound drug is not cleared; Cl = free fraction × GFR

STEADY STATE

Steady state is reached either when **rate in = rate out** or when values associated with a dosing interval are the same as those in the succeeding interval.

Plateau Principle

High-Yield

The time to reach steady state is dependent only on the elimination half-life of a drug. It is independent of dose size and frequency of administration, assuming the drug is eliminated by first-order kinetics.

Note

$$\text{Maintenance dose} = \frac{C^{ss} \times Cl \times \tau}{\text{Bioavailability}}$$

The figure below shows plasma levels (solid lines) achieved following the IV bolus administration of 100 units of a drug at intervals equivalent to every **half-life** $\mathbf{t_{1/2}}$ **= 4 h** (τ). With such intermittent dosing, plasma levels oscillate through peaks and troughs, with averages shown in the diagram by the dashed line.

Classic Clues

Time and Steady State

50% = 1 × half-life

90% = 3.3 × half-life

95% = 4–5 × half-life

“100”% = >7 × half-life

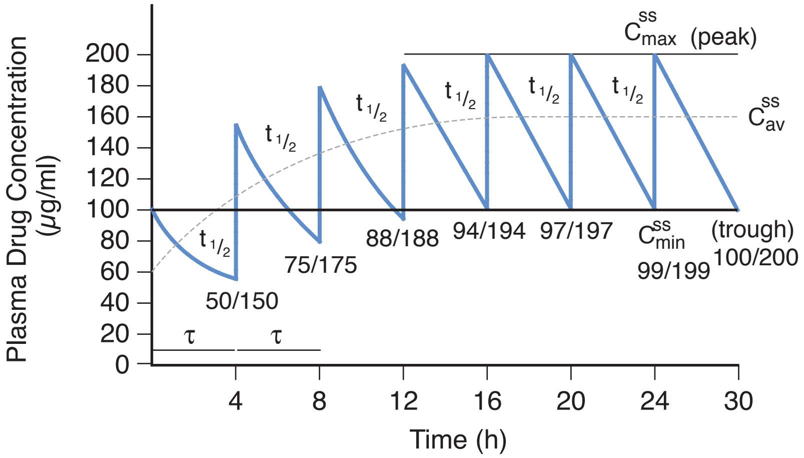


Figure I-1-11. Oscillations in Plasma Levels following IV Bolus Administration at Intervals Equal to Drug Half-Life

Note: Although it takes >7 $t_{1/2}$ to reach **mathematical** steady state, by convention **clinical** steady state is accepted to be reached at 4–5 $t_{1/2}$.

Rate of Infusion

High-Yield

The figure below shows the increase in plasma level of the same drug infused at five rates. Regardless of the rate of infusion, it takes the same amount of time to reach steady state.

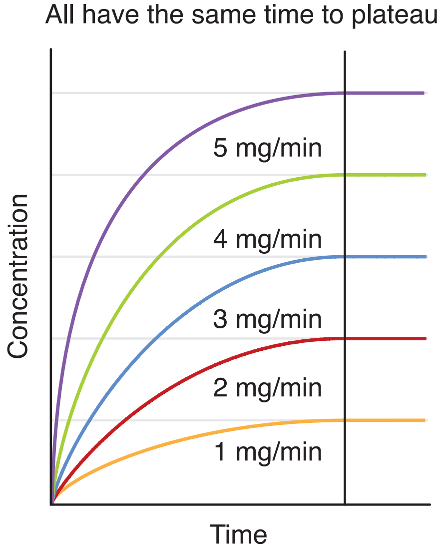


Figure I-1-12. Effect of Rate of Infusion on Plasma

Rate of infusion (k_0) does determine plasma level at steady state. If the rate of infusion is doubled, then the plasma level of the drug at steady state is doubled. A similar relationship can exist for other forms of drug administration (e.g., per oral)—doubling oral doses can double the average plasma levels of a drug. Plotting dose against plasma concentration yields a straight line (linear kinetics).

Effect of Loading Dose

High-Yield

It takes 4–5 half-lives to achieve steady state. In some situations, a higher dose (loading dose) may be needed to more rapidly achieve effective blood levels (C_p).

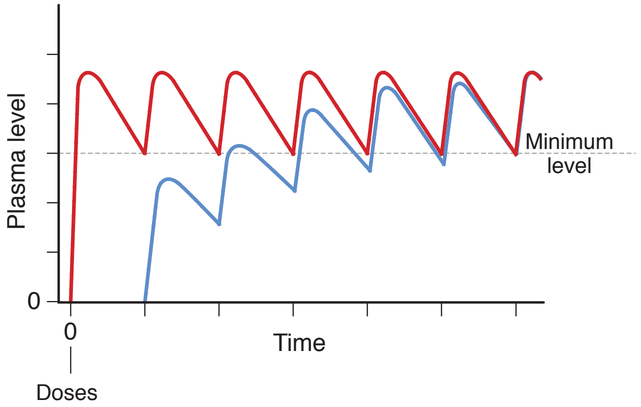


Figure I-1-13. Effect of a Loading Dose on the Time Required to Achieve the Minimal Effective Plasma Concentration

Note

Dose and plasma concentration (C^{SS}) are directly proportional.

Note

$$\text{Loading dose} = \frac{V_d \times C_p}{f}$$

Clinical Correlate

The loading dose equation can be used to calculate the amount of drug in the body at any time by knowing the V_d and plasma concentration.

Note

For the exam, if doses are to be administered at each half-life of the drug, and minimum effective concentration is equivalent to C^{SS}_{min}, then the loading dose is twice the amount of the dose used for maintenance (assuming normal clearance and same bioavailability for maintenance doses).

For any other interval of dosing, use the equation listed in the Note above.

C^0 = conc. at time zero

Cl = clearance

C_p = conc. in plasma

C^{ss} = steady state conc.

D = dose

f = bioavailability

τ = dosing interval

Such loading doses are often one time only and are estimated to put into the body the amount of drug that should be there at a steady state.

PHARMACOKINETICS CALCULATIONS

The following relationships are important for pharmacokinetic calculations:

Single-Dose Equations

Volume of distribution $(V_d) = \frac{D}{C^0}$

Half-life $(t_{1/2}) = 0.7 \times \frac{V_d}{Cl}$

Multiple Dose or (Infusion Rate) Equations

Infusion rate $(k_0) = Cl \times C^{ss}$

Loading dose $(LD) = \frac{V_d \times C_p}{f}$

Maintenance dose $(MD) = \frac{Cl \times C^{ss} \times \tau}{f}$

Pharmacodynamics 2

Learning Objectives

- ❑ Differentiate between graded (quantitative) dose-response (D-R), and quantal (cumulative) D-R curves
- ❑ Use knowledge of signaling mechanisms
- ❑ Demonstrate understanding of drug development and testing

DEFINITIONS

Pharmacodynamics relates to drugs binding to receptors and their effects.

- A drug is called an **agonist** when binding to the receptor results in a response.
- A drug is called an **antagonist** when binding to the receptor is not associated with a response; the drug has an effect only by preventing an agonist from binding to the receptor.
- **Affinity** is the ability of a drug to bind to receptor, shown by the proximity of the curve to the *y* axis (if the curves are parallel); the nearer the *y* axis, the greater the affinity.
- **Potency** shows relative doses of ≥2 agonists to produce the same magnitude of effect, again shown by the proximity of the respective curves to the *y* axis (if the curves do not cross).
- **Efficacy** is a measure of how well a drug produces a response (effectiveness), shown by the maximal height reached by the curve.

Bridge to Biochemistry

Affinity is how well a drug and a receptor recognize each other.

- Inversely related to K_d of the drug
- Notice analogy to K_m value used in enzyme kinetic studies

Potency is the quantity of drug required to achieve a desired effect.

- In D-R measurements, the chosen effect is usually 50% of maximal effect but clinically any size response can be sought.

Efficacy is the maximal effect an agonist can achieve at the highest practical concentration.

- Notice analogy to V_{max} used in enzyme kinetic studies

GRADED (QUANTITATIVE) DOSE-RESPONSE (D-R) CURVES

Plots of dose (or log dose) versus response for drugs (**agonists**) that activate receptors can reveal information about affinity, potency, and efficacy of these agonists.

Parallel and Nonparallel D-R Curves

High-Yield

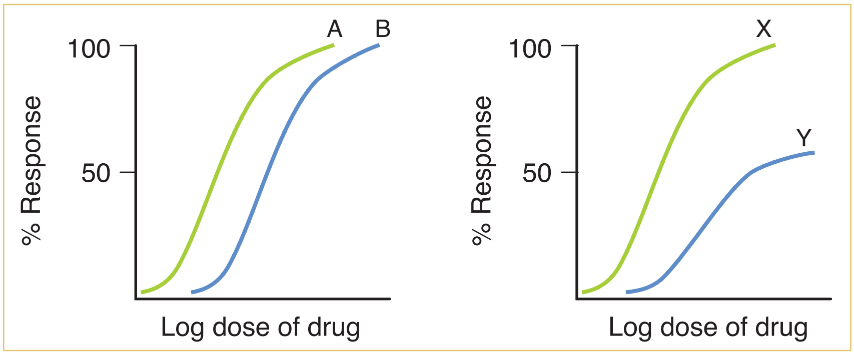


Figure I-2-1. D-R Curves for 2 Drugs Acting on Same (left) and Different (right) Receptors

It may be seen from the log dose-response curves above that:

- When 2 drugs interact with the same receptor (same pharmacologic mechanism), the D-R curves will have parallel slopes. Drugs A and B have the same mechanism; drugs X and Y do not.
- Affinity can be compared only when 2 drugs bind to the same receptor. Drug A has a greater affinity than drug B.
- In terms of potency, drug A has greater potency than drug B,
- and X is more potent than Y.
- In terms of efficacy, drugs A and B are equivalent. Drug X has greater efficacy than drug Y.

Full and Partial Agonists

Full agonists produce a maximal response, i.e., they have maximal efficacy. Partial agonists are less effective, i.e., they are incapable of eliciting a maximal response.

In the figure below, drug B is a full agonist while drugs A and C are partial agonists.

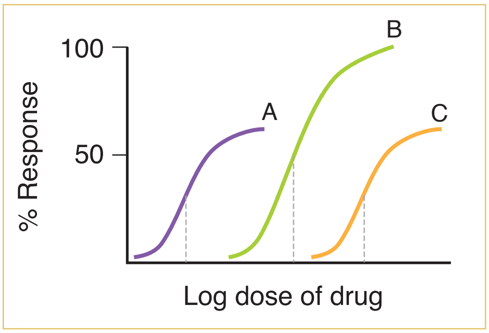


Figure I-2-2. Efficacy and Potency of Full and Partial Agonists

- Drug A is more potent than drug C, and drug B is more potent than drug C.
- However, no general comparisons re potency can be made between drugs A and B because the former is a partial agonist and the latter is a full agonist.
- At low responses, A is more potent than B, but at high responses, the reverse is true.

Duality of Partial Agonists

In the figure below, the lower curve represents effects of a partial agonist when used alone; its **ceiling effect** is 50% of maximal in this example.

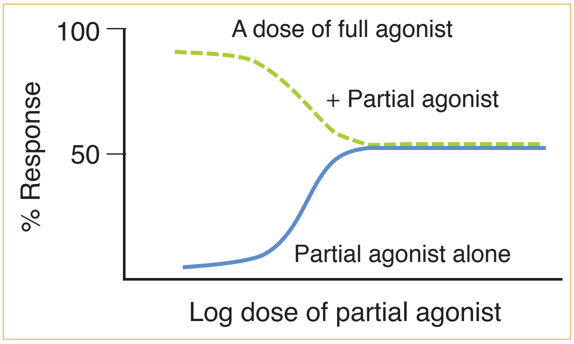


Figure I-2-3. Duality of Partial Agonists

- The upper curve shows the effect of increasing doses of the partial agonist on the maximal response (100%) achieved in the presence of or by pretreatment with a full agonist.
- As the partial agonist displaces the full agonist from the receptor, the response is reduced—the partial agonist is acting as an **antagonist**.

Antagonism and Potentiation

Graded dose-response curves also provide information about antagonists (drugs that interact with receptors to interfere with their activation by agonists).

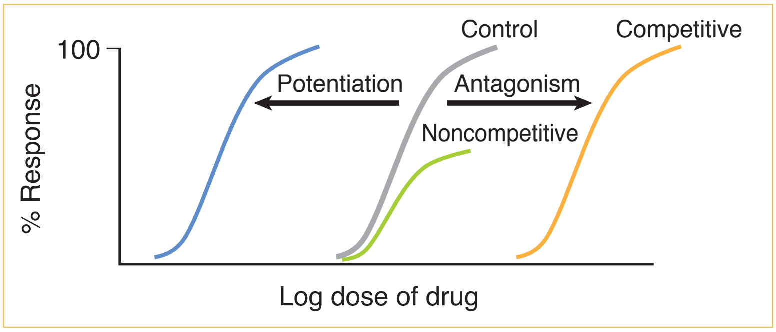


Figure I-2-4. D-R Curves of Antagonists and Potentiators

Bridge to Biochemistry

Parallels between Receptor Antagonists and Enzyme Inhibitors

Competitive antagonists are analogous to competitive inhibitors; they decrease affinity (↑ K_m) but not maximal response (V_{max} remains the same).

Noncompetitive antagonists decrease V_{max} but do not change the K_m.

- Pharmacologic antagonism (same receptor)
  - Competitive antagonists (cause parallel shift to the right in D-R curve for agonists)
    - Can be reversed by increasing dose of agonist drug
    - Appear to decrease potency of agonist
  - Noncompetitive antagonists (cause nonparallel shift to the right)
    - Can be only partially reversed by increasing dose of agonist
    - Appear to decrease efficacy of the agonist
- Physiologic antagonism (different receptor)
  - Two agonists with opposing action antagonize each other
  - Example: a vasoconstrictor with a vasodilator
- Chemical antagonism:
  - Formation of a complex between effector drug and another compound
  - Example: protamine binds to heparin to reverse its actions
- Potentiation
  - Causes a parallel shift to the left to the D-R curve
  - Appears to increase potency of agonist

QUANTAL (CUMULATIVE) D-R CURVES

Quantal D-R curves plot the percentage of a population responding to a specified drug effect versus dose or log dose. They permit estimations of the median effective dose, or effective dose in 50% of a population—ED50.

- Can reveal the range of intersubject variability in drug response
- Steep D-R curve reflects little variability
- Flat D-R curve indicates great variability in patient sensitivity to the effects of a drug

Toxicity and Therapeutic Index

Comparisons between ED50 and TD50 values permit evaluation of the relative safety of a drug (the therapeutic index, or TI), as would comparison between ED50 and the lethal median dose (LD50) if the latter is known.

$$TI = \frac{TD50}{ED50} \text{ or } \frac{LD50}{ED50}$$

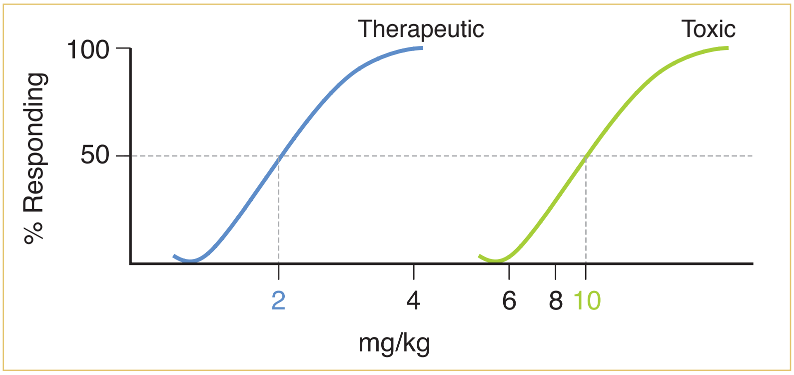


Figure I-2-5. Quantal D-R Curves of Therapeutic and Toxic Effects of a Drug

D-R curves can also show the relationship between dose and toxic effects of a drug. The median toxic dose of a drug (TD50) is the dose that causes toxicity in 50% of a population. From the data above, TI = 10/2 = 5.

Such indices are of most value when toxicity represents an extension of the pharmacologic actions of a drug. They do not predict idiosyncratic reactions or drug hypersensitivity.

Recall Question

Which of the following best describes the effect of a competitive antagonist on the dose-response curve?

A. Non-parallel left shift

B. Non-parallel right shift

C. Parallel left shift

D. Parallel right shift

Answer: D

SIGNALING MECHANISMS

The binding of an agonist drug to its receptor activates an effector or **signaling mechanism**. Several types of drug-responsive signaling mechanisms are known.

Intracellular Receptors

Intracellular receptors include receptors for steroids. Binding of hormones or drugs to such receptors releases regulatory proteins which permit activation (and in some cases, dimerization) of the hormone-receptor complex.

- Such complexes translocate to the nucleus, where they interact with response elements in spacer DNA. This interaction leads to changes in gene expression.
- For example, drugs interacting with glucocorticoid receptors lead to gene expression of proteins that inhibit the production of inflammatory mediators.

Other examples of intracellular receptors include intracellular receptors for thyroid hormones, gonadal steroids, and vitamin D.

Pharmacologic responses elicited via modification of gene expression are usually slower in onset but longer in duration than many other drugs.

Membrane Receptors Directly Coupled to Ion Channels

Many drugs act by mimicking or antagonizing the actions of endogenous ligands that regulate flow of ions through excitable membranes via their activation of receptors that are directly coupled (no second messengers) to ion channels.

- For example, the nicotinic receptor for ACh (present in autonomic nervous system [ANS] ganglia, the skeletal myoneural junction, and the central nervous system [CNS]) is coupled to a Na+/K+ ion channel. The receptor is a target for many drugs, including nicotine, choline esters, ganglion blockers, and skeletal muscle relaxants.
- Similarly, the $GABA_A$ receptor in the CNS, which is coupled to a chloride ion channel, can be modulated by anticonvulsants, benzodiazepines, and barbiturates.

Receptors Linked Via Coupling Proteins to Intracellular Effectors

Many receptor systems are coupled via GTP-binding proteins (G proteins) to adenyl cyclase, the enzyme that converts ATP to cAMP, a second messenger which promotes protein phosphorylation by activating protein kinase A. These receptors are typically "serpentine," with 7 transmembrane spanning domains, a third of which is coupled to the G-protein effector mechanism.

- Protein kinase A serves to phosphorylate a set of tissue-specific substrate enzymes or transcription factors (CREB), thereby affecting their activity.

Note

Key ANS Receptors

M_1, M_3, α_1: G_q activation of phospholipase C

M_2, α_2, D_2: G_i inhibition of adenylyl cyclase

β_1, β_2, D_1: G_s activation of adenylyl cyclase

G_s proteins

- Binding of agonists to receptors linked to G_s proteins increases cAMP production.
- Such receptors include those for catecholamines (beta), dopamine (D_1), glucagon, histamine (H_2), prostacyclin, and some serotonin subtypes.

G_i proteins

- Binding of agonists to receptors linked to G_i proteins decreases cAMP production.
- Such receptors include adrenoreceptors ($alpha_2$), ACh (M_2), dopamine (D_2 subtypes), and several opioid and serotonin subtypes.

G_q proteins

Other receptor systems are coupled via GTP-binding proteins (G_q), which activate phospholipase C. Activation of this enzyme releases the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG) from the membrane phospholipid phosphatidylinositol bisphosphate (PIP_2). The IP_3 induces release of

Ca^{2+} from the sarcoplasmic reticulum (SR), which, together with DAG, activates protein kinase C. The protein kinase C serves then to phosphorylate a set of tissue-specific substrate enzymes, usually not phosphorylated by protein kinase A, and thereby affects their activity.

- These signaling mechanisms are invoked following activation of receptors for ACh (M_1 and M_3), norepinephrine ($alpha_1$), angiotensin II, and several serotonin subtypes.

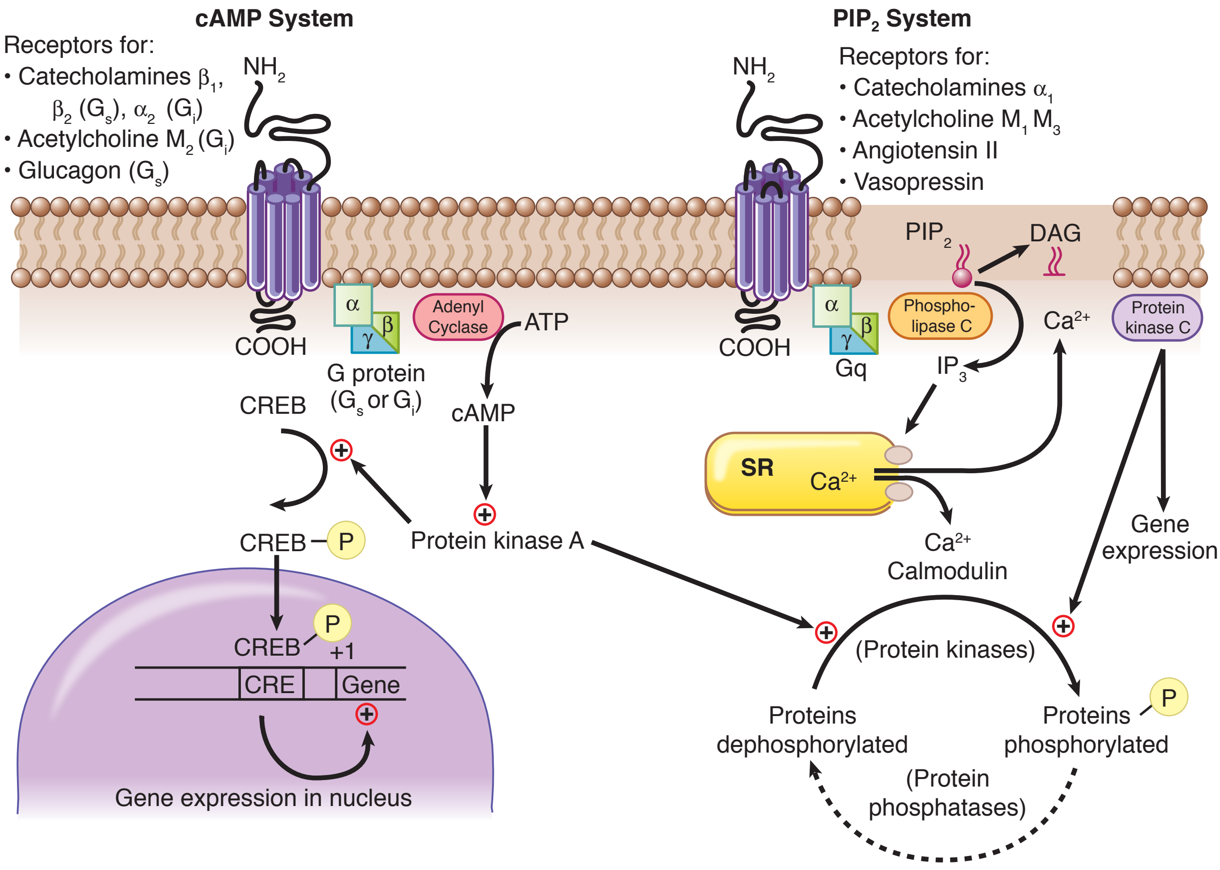


Figure I-2-6. Receptors Using Cyclic AMP and IP_3 , DAG, Ca^{2+} as Second Messengers

Cyclic GMP and Nitric Oxide Signaling

Cyclic GMP (cGMP) is a second messenger in vascular smooth muscle that facilitates dephosphorylation of myosin light chains, preventing their interaction with actin and thus causing vasodilation. Nitric oxide (NO) is synthesized in endothelial cells and diffuses into smooth muscle.

- NO activates guanylyl cyclase, thus increasing cGMP in smooth muscle.
- Vasodilators ↑ synthesis of NO by endothelial cells.

Bridge to Biochemistry

See Chapter 9 of the Biochemistry Lecture Notes for additional discussion of signal transduction.

Clinical Correlate

Drugs acting via NO include nitrates (e.g., nitroglycerin) and M-receptor agonists (e.g., bethanechol). Endogenous compounds acting via NO include bradykinin and histamine.

Receptors That Function as Enzymes or Transporters

There are multiple examples of drug action which depends on enzyme inhibition, including inhibitors of acetylcholinesterase, angiotensin-converting enzyme, aspartate protease, carbonic anhydrase, cyclooxygenases, dihydrofolate reductase, DNA/RNA polymerases, monoamine oxidases, Na/K-ATPase, neuraminidase, and reverse transcriptase.

- Examples of drug action on transporter systems include the inhibitors of reuptake of several neurotransmitters, including dopamine, GABA, norepinephrine, and serotonin.

Receptors That Function as Transmembrane Enzymes

- These receptors mediate the first steps in signaling by insulin and growth factors, including epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). They are membrane-spanning macromolecules with recognition sites for the binding of insulin and growth factors located externally and a cytoplasmic domain that usually functions as a tyrosine kinase. Binding of the ligand causes conformational changes (e.g., dimerization) so that the tyrosine kinase domains become activated, ultimately leading to phosphorylation of tissue-specific substrate proteins.
- Guanyl cyclase–associated receptors: stimulation of receptors to atrial natriuretic peptide activates the guanyl cyclase and ↑ cyclic GMP (cGMP)

Clinical Correlate

Imatinib is a specific tyrosine-kinase (TK) inhibitor, while sorafenib is a non-specific TK inhibitor.

Receptors for Cytokines

Receptors for cytokines include the receptors for erythropoietin, somatotropin, and interferons. Their receptors are membrane spanning, and on activation, can activate a distinctive set of cytoplasmic tyrosine kinases (Janus kinases [JAKs]).

- JAKs phosphorylate signal transducers and activators of transcription (STAT) molecules.
- STATs dimerize and then dissociate, cross the nuclear membrane, and modulate gene transcription.

Practice Questions 3

PRACTICE QUESTIONS

1. A patient was given a 200 mg dose of a drug IV, and 100 mg was eliminated during the first 2 hours. If the drug follows first-order elimination kinetics, how much of the drug will remain 6 hours after its administration?

   A. None
   B. 25 mg
   C. 50 mg
   D. 75 mg
   E. 100 mg

2. Drugs that are administered IV are

   A. Rapidly absorbed
   B. Subject to first-pass metabolism
   C. 100% bioavailable
   D. Rapidly excreted by the kidneys
   E. Rapidly metabolized by the liver

3. Drugs that are highly bound to albumin:

   A. Effectively cross the BBB
   B. Are easily filtered at the glomerulus
   C. Have a large V_d
   D. Often contain quaternary nitrogens
   E. Can undergo competition with other drugs for albumin binding sites

4. Most drugs gain entry to cells by:

   A. Passive diffusion with zero-order kinetics
   B. Passive diffusion with first-order kinetics
   C. Active transport with zero-order kinetics
   D. Active transport with first-order kinetics
   E. Passive diffusion through membrane pores

5. A subject in whom the renal clearance of inulin is 120 mL/min is given a drug, the clearance of which is found to be 18 mL/min. If the drug is 40% plasma protein bound, how much filtered drug must be reabsorbed in the renal tubules?

   A. None
   B. 18 mL/min
   C. 36 mL/min
   D. 54 mL/min
   E. 72 mL/min

6. If a drug is known to be distributed into total body water, what dose (mg) is needed to obtain an initial plasma level of 5 mg/L in a patient weighing 70 kg?

   A. 210
   B. 150
   C. 110
   D. 50
   E. 35

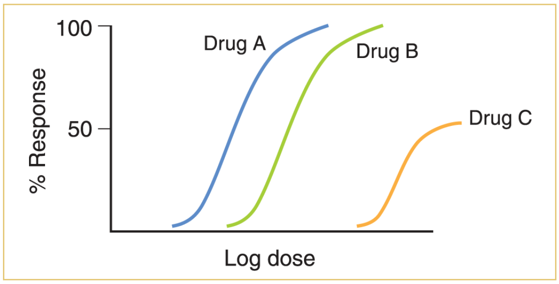
7. Which of the following is a phase II drug metabolism reaction associated with a genetic polymorphism?

   A. Acetylation
   B. Glucuronidation
   C. Oxidation
   D. Reduction
   E. Glutathione conjugation

8. A woman is taking oral contraceptives (OCs). Which of the following drugs is unlikely to reduce the effectiveness of the OCs?

   A. Carbamazepine
   B. Phenytoin
   C. Ketoconazole
   D. Phenobarbital
   E. Rifampin

9. The data presented in the figure below show that:



A. Drugs A and B have equal efficacy
B. Drug B and C have equal efficacy
C. Drug B is a partial agonist
D. Drugs A and C have the same affinity and efficacy
E. Drugs A and B have equal potency

10. A 500-mg dose of a drug has therapeutic efficacy for 6 h. If the half-life of the drug is 8 h, for how long would a 1-g dose be effective?

A. 8 h
B. 12 h
C. 14 h
D. 16 h
E. 24 h

11. Which statement is accurate for the drug shown in the example below?

100 mg $^{2hr} \rightarrow$ 50 mg $^{2hr} \rightarrow$ 25 mg $^{2hr} \rightarrow$ 12.5 mg

A. The rate of elimination is constant
B. The elimination half-life varies with the dose
C. The volume of distribution varies with the dose
D. The clearance varies with the dose
E. The rate of elimination varies directly with the dose

12. Normally, acetaminophen has a V_d = 70L and C1 = 350 mL/min. If acetaminophen was administered to a patient with 50% renal function, what parameter would differ from normal?

A. Loading dose would be higher
B. Maintenance dose would be lower
C. t ½ would be shorter
D. V_d would be 35L
E. Cl would be 700 mL/min

13. Pharmacokinetic characteristics of propranolol include $V_d = 300$ L/70 kg, C1 = 700 mL/min, and oral bioavailability f = 0.25. What is the dose needed to achieve a plasma level equivalent to a steady-state level of 20 μg/L?

    A. 4 mg
    B. 8 mg
    C. 12 mg
    D. 24 mg
    E. 48 mg

14. With IV infusion, a drug reaches 50% of its final steady state in 6 hours. The elimination half-life of the drug must be approximately:

    A. 2 h
    B. 6 h
    C. 12 h
    D. 24 h
    E. 30 h

15. At 6 h after IV administration of bolus dose, the plasma level of a drug is 5 mg/L. If the $V_d = 10$ L and the elimination half-life = 3 h, what was the dose administered?

    A. 100 mg
    B. 150 mg
    C. 180 mg
    D. 200 mg
    E. 540 mg

16. An IV infusion of a drug is started 400 mg/h. If C1 = 50 L/h, what is the anticipated plasma level at steady state?

    A. 2 mg/L
    B. 4 mg/L
    C. 8 mg/L
    D. 16 mg/L
    E. 32 mg/L

ANSWERS AND EXPLANATIONS

1. **Answer: B.** One half of the dose is eliminated in the first 2 hours so its elimination half-life equals 2 hours. With the passage of each half-life the amount in the body (or in the blood) will decrease to 50% of a former level. Thus, at 6 hours after administration, 3 half-lives have passed: (1) 200 mg to 100 mg, (2) 100 mg to 50 mg, and (3) 50 mg to 25 mg.

2. **Answer: C.** By definition, IV administration does not involve absorption because there is no movement from the site of administration into the blood. The IV route avoids first-pass metabolism which is common with orally administered drugs. First-pass greatly reduces the bioavailability of many drugs. Drugs given IV have 100% bioavailability ($f = 1$) since the entire dose is in the systemic circulation. No conclusions can be drawn about renal or hepatic elimination of a drug knowing only that it was administered IV.

3. **Answer: E.** Since most drugs are lipid-soluble they will need a carrier in the blood, most commonly albumin. Drugs bound to albumin do not get filtered at the glomerulus or cross the blood-brain barrier. Binding to plasma proteins keeps drugs in the plasma resulting in a lower V_d. Highly protein bound drugs are good candidates for interactions with other drugs that are also highly bound (e.g., warfarin plus sulfonamides).

4. **Answer: B.** The permeation of most drugs through cellular membranes is by the process of passive diffusion, a nonsaturable process that follows first-order kinetics. Concentration gradient and lipid solubility are important determinants for the rate of diffusion. Only a few drugs are substrates for active transport processes such as active tubular secretion (e.g., penicillins) or penetrate membranes via aqueous pores (ethanol).

5. **Answer: D.** The formula to use is $Cl = ff \times GFR$. The drug is 40% protein bound so the $ff = 60\%$. $120 \text{ mL/min} \times 60\% = 72 \text{ mL/min}$ theoretical clearance of the drug. Since only 18 mL/min was actually cleared, there must have been tubular reabsorption of the drug. $72 - 18 = 54$ mL/min of reabsorbed drug.

6. **Answer: A.** This is a "loading dose" question. The equation for loading dose or the volume of distribution equation can be used ($LD = V_d \times C_p$). Since the patient weighs 70 kg and 60% of body weight is water, he has 42 L (70 L $\times$ 60%) of total body water. $LD = 42 \text{ L} \times 5 \text{ mg/L} = 210 \text{ mg}$.

7. **Answer: A.** Phase II drug metabolism involves the transfer of chemical groupings (e.g. acetyl, glucuronide, glutathione) to drugs or their metabolites via conjugation reactions involving transferase enzymes. Acetylation reactions are associated with a genetic polymorphism (slow acetylator). These individuals are slow to metabolize drugs via acetylation and are particularly susceptible to drug-induced SLE when taking hydralazine, procainamide, or isoniazid. Both oxidation and reduction are phase I metabolism reactions.

8. **Answer: C.** Azole antifungals (e.g. ketoconazole) are inhibitors of cytochrome P450 enzymes, especially CYP3A4, the most abundant isozyme form in the human liver. The 3A4 isozyme metabolizes a wide range of drugs. Ketoconazole would actually raise the plasma levels of oral contraceptives increasing the risk of side effects but it would not reduce their effectiveness. All other drugs listed are P450 inducers. As such, they would tend to lower plasma levels and decrease effectiveness of oral contraceptives.

9. **Answer: A.** The typical log dose response figure with the parallel nature of the curves suggests that the three drugs are interacting with the same receptor system. Drugs A and B are full agonists because they achieve the maximal response. They have the same efficacy. Drug A is more potent than drugs B or C. Drug B is more potent than drug C. Drug C is a partial agonist with less efficacy than the full agonists.

10. **Answer: C.** The fact that the drug has therapeutic efficacy for 6 h has no direct relationship to its half-life—it simply means that the drug is above its minimal effective concentration for 6 h. Doubling the dose (to 1 g) means that the drug level will be above the minimum for a longer period. Because the elimination half-life is 8 h, 500 mg of the drug will remain in the body 8 h after a dose of 1 g. Thus, the total duration of effectiveness must be 8 + 6 = 14 h.

11. **Answer: E.** In first-order kinetics, the elimination rate of a drug is directly proportional to its plasma concentration, which in turn is proportional to the dose. Drugs that follow first-order elimination have a constant elimination half-life similar to the example given in the question. Likewise, clearance and volume of distribution are pharmacokinetic characteristics of a drug that do not routinely change with dose, although they may vary in terms of disease or dysfunction.

12. **Answer: E.** The patient has renal dysfunction which reduces renal clearance. This would necessitate a lower maintenance dose for medications such as acetaminophen. The maintenance dose equation $\left(MD = \frac{Cl \times C^{ss} \times}{f}\right)$ factors in renal clearance while the loading dose equation does not. The t ½ of acetaminophen would be increased in this patient due to the decrease in clearance, but the V_d would be unaffected.

13. **Answer: D.** Loading dose = $V_d \times \frac{C_p}{f}$ LD = 300L × 20 µg/L ÷ 0.25

= 6000 µg/0.25 = 24,000 µg or 24 mg

14. **Answer: B.** The rules for time to steady-state are that it takes 4–5 t ½ to reach clinical steady-state. It also takes one t ½ to get half way to steady-state. Since the drug got 50% of the way to steady-state in 6 hours, its t ½ must be 6 hours.

15. **Answer: D.** At 6 h after IV injection (which corresponds to two half-lives of the drug), the plasma level is 5 mg/L. Extrapolating back to zero time, "doubling" plasma level for each half-life results in an initial plasma level at zero time (C^0) = 5 mg/L × 2 × 2 = 20 mg/L.

$$\begin{aligned} \text{Dose} &= C^0 \times V_d \\ &= 20 \text{ mg/L} \times 10 \text{ L} \\ &= 200 \text{ mg} \end{aligned}$$

16. **Answer: C.** $\text{MD} = \text{Cl} \times C^{ss} \times \tau$

Since the drug was given by constant IV infusion there is no need to consider the dosing interval (τ). Therefore, $400 \text{ mg/h} = 50 \text{ L/h} \times C^{ss}$

$$400 \text{ mg/h} \div 50 \text{ L/h} = 8 \text{ mg/L}$$

Alternatively, you could evaluate the question this way:

An infusion rate (k_0) is given by:

$$k_0 = \text{Cl} \times C^{ss}$$

$$\text{rearrange: } C^{ss} = k_0/\text{Cl}$$

$$= \frac{400 \text{ mg/h}}{50 \text{ L/h}} = 8 \text{ mg/L}$$

PART II

Autonomic Pharmacology

The Autonomic Nervous System 1

Learning Objectives

- ❑ Explain information related to anatomy of the ANS
- ❑ Solve problems concerning blood pressure control mechanisms
- ❑ Answer questions related to pupillary size and accommodation mechanisms

ANATOMY OF THE ANS

The autonomic nervous system (ANS) is the major involuntary portion of the nervous system, and is responsible for automatic, unconscious bodily functions (e.g., control of heart rate and blood pressure, and both gastrointestinal and genitourinary functions). It is divided into two subcategories: the **parasympathetic autonomic nervous system** (PANS) and the **sympathetic autonomic nervous system** (SANS).

Location of ANS Ganglia

Both the PANS and SANS have relay stations, or ganglia, between the CNS and the end organ, but the somatic system does not. An important anatomic difference between them is that the ganglia of the SANS lie in 2 paraventral chains adjacent to the vertebral column, whereas most of the ganglia of the PANS system are located in the organs innervated.

ANS and Somatic Innervation

High-Yield

The figure below highlights the major features of the ANS and the somatic systems, and also shows the location of the major receptor types.

- **N_N** Nicotinic receptors are located on cell bodies in ganglia of both PANS and SANS and in the adrenal medulla.
- **N_M** Nicotinic receptors are located on the skeletal muscle motor end plate innervated by somatic motor nerves.
- **M_{1-3}** Muscarinic receptors are located on all organs and tissues innervated by postganglionic nerves of the PANS and on thermoregulatory sweat glands innervated by the SANS.

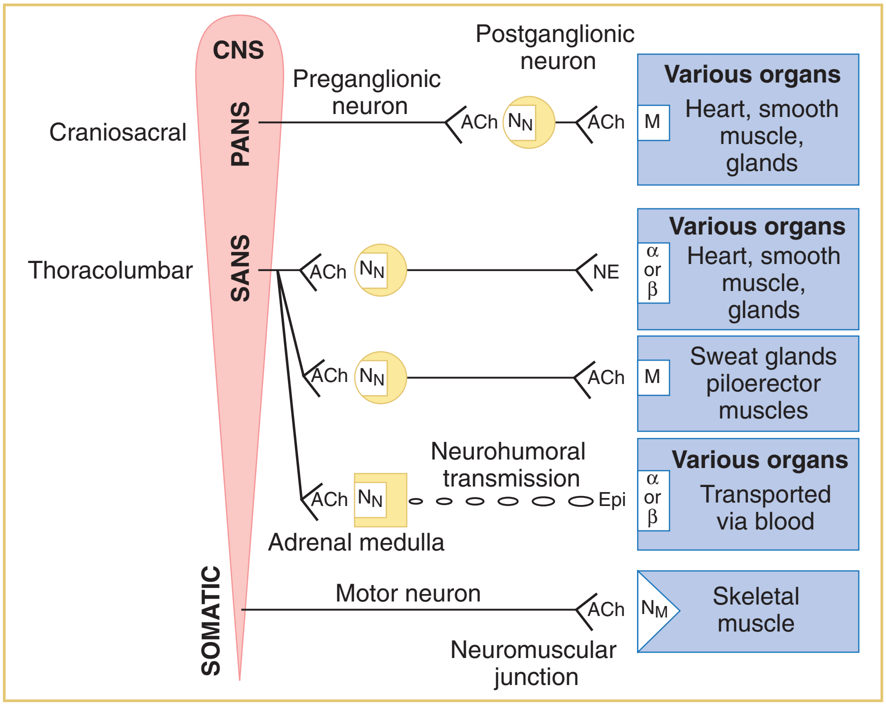


Figure II-1-1. Anatomy of the Autonomic Nervous System

Neurotransmitters

- Acetylcholine (ACh) is the neurotransmitter at both nicotinic and muscarinic receptors in tissues that are innervated. Note that all direct transmission from the CNS (preganglionic and motor) uses ACh, but postganglionic transmission in the SANS system may use one of the organ-specific transmitters described below.
- Norepinephrine (NE) is the neurotransmitter at most adrenoceptors in organs, as well as in cardiac and smooth muscle.
- Dopamine (DA) activates D_1 receptors, causing vasodilation in renal and mesenteric vascular beds.
- Epinephrine (E, from adrenal medulla) activates most adrenoceptors and is transported in the blood.

BLOOD PRESSURE CONTROL MECHANISMS

Autonomic Feedback Loop

High-Yield

Blood pressure (BP) is the product of total peripheral resistance (TPR) and cardiac output (CO). Both branches of the ANS are involved in the autonomic (or neural) control of blood pressure via feedback mechanisms.

- Changes in mean BP are detected by baroreceptors, which relay information to the cardiovascular centers in the brainstem controlling PANS and SANS outflow.
  - For example, an increase in mean BP elicits baroreceptor discharge, resulting in increased PANS activity, leading to bradycardia and

decreased SANS activity, which leads, in turn, to decreased heart rate, force of contraction, and vasoconstriction. The resulting decreases in cardiac output and total peripheral resistance contribute to restoration of mean BP toward its normal level.

– Conversely, a decrease in BP elicits ANS neural feedback involving decreased PANS outflow and increased SANS activity—actions leading to an increase in cardiac output and total peripheral resistance.

Note

Baroreceptor reflexes can be blocked at the ganglionic synapse with N_N receptor antagonists. Alternatively, a reflex bradycardia can be blocked with muscarinic antagonists; a reflex tachycardia can be blocked with β_1 antagonists.

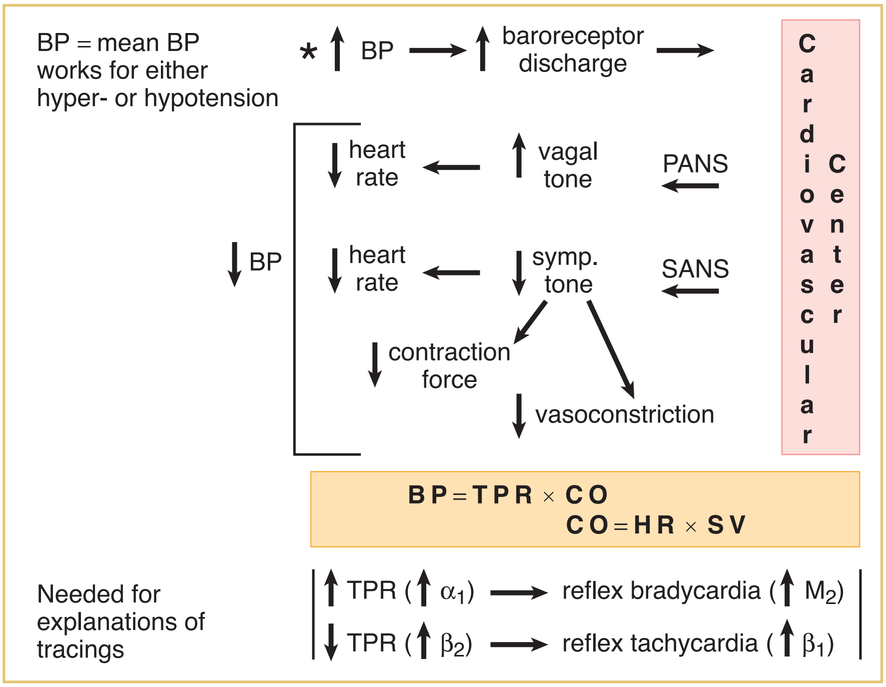


Figure II-1-2. Autonomic Feedback Loop

Hormonal Feedback Loop

Blood pressure is also regulated via the hormonal feedback loop. The system is affected only by **decreases** in mean BP (hypotension), which results in decreased renal blood flow.

- Decreased renal pressure causes the release of renin, which promotes formation of the angiotensins.
- Angiotensin II increases aldosterone release from the adrenal cortex, which, via its mineralocorticoid actions to retain sodium and water, increases blood volume.
- Increased venous return results in an increase in cardiac output.
- Angiotensin II also causes vasoconstriction, resulting in an increase in TPR.

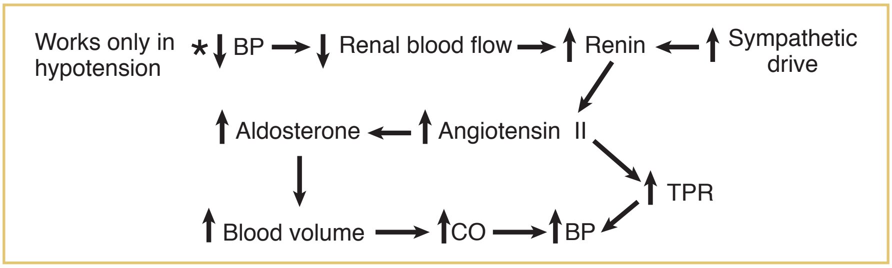


Figure II-1-3. Hormonal Feedback Loop

Blood Pressure/Heart Rate Tracings

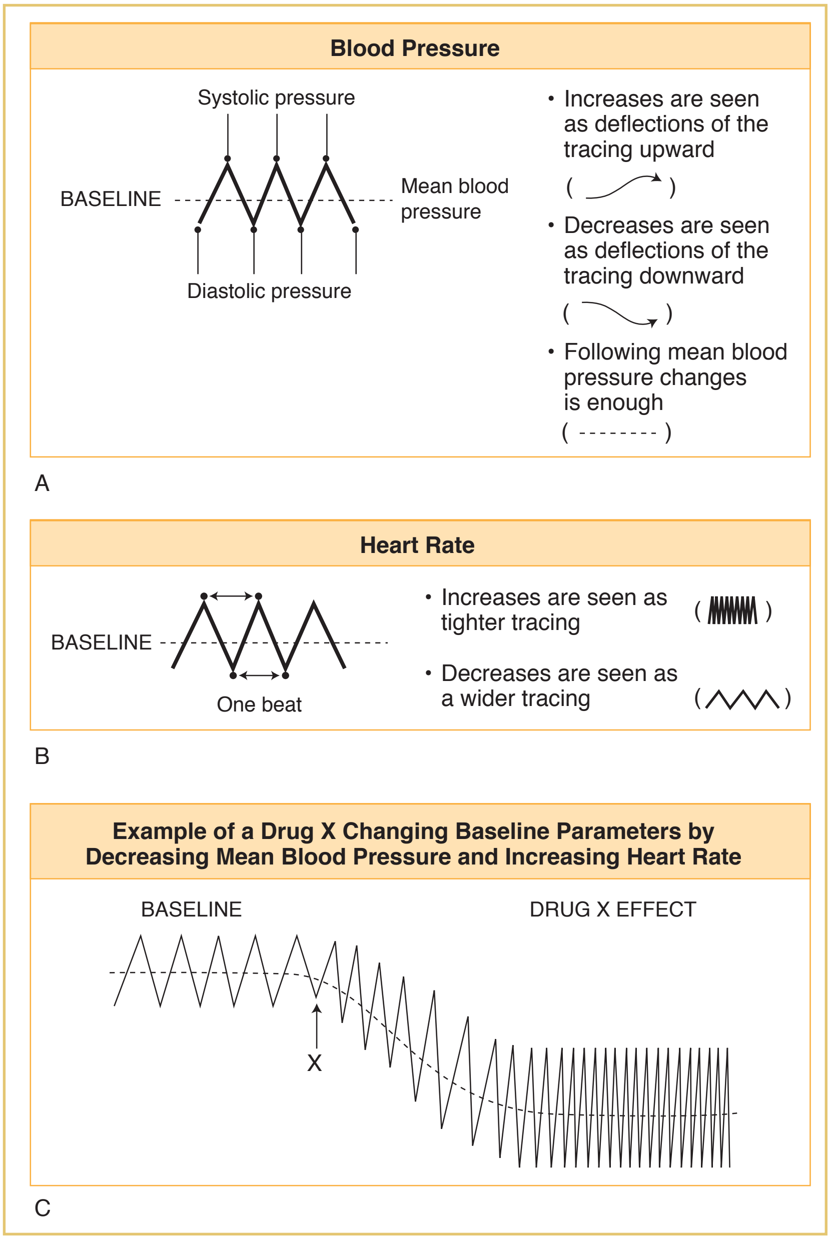


Figure II-1-4. Blood Pressure/Heart Rate Tracings

Note

Both the ANS (neural) and endocrine feedback loops are invoked when patients are treated with **antihypertensive drugs.** Such compensatory mechanisms may result in tachycardia and both salt and water retention.

PUPILLARY SIZE AND ACCOMMODATION MECHANISMS

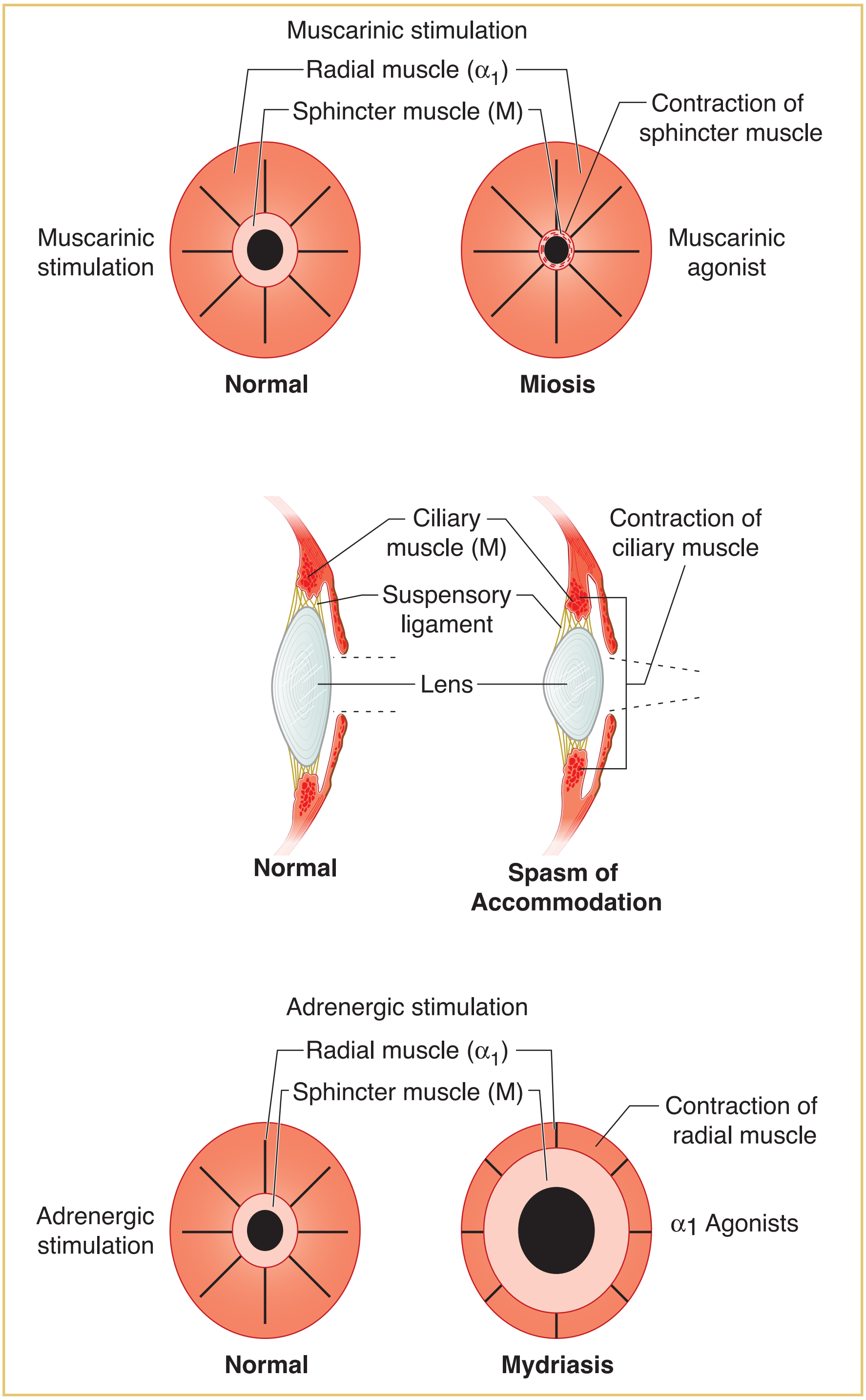


Figure II-1-5. Effect of ANS Drugs on the Eye

Muscarinic stimulation

1. Miosis
2. Accommodation (near vision)

Muscarinic antagonism

1. Mydriasis
2. Accommodation to far vision, leading to cycloplegia (paralysis of accommodation)

α1-agonists

1. Mydriasis
2. No cycloplegia

Cholinergic Pharmacology 2

Learning Objectives

- ❑ Answer questions about cholinergic neuroeffector junctions
- ❑ Differentiate between muscarinic receptor activators, receptor antagonists, and nicotinic receptor antagonists

CHOLINERGIC NEUROEFFECTOR JUNCTIONS

Synthesis and Release of ACh

High-Yield

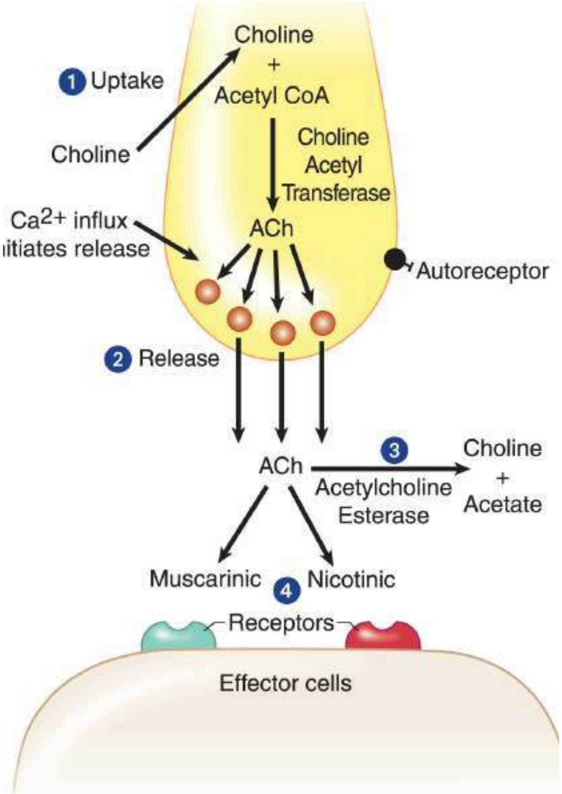


Figure II-2-1. Cholinergic Neuroeffector Junction

1 Hemicholinium

2 Botulinum toxin

3 Acetylcholinesterase (AChE) inhibitors

4 Receptor agonists and antagonists

Choline is accumulated in cholinergic presynaptic nerve endings via an active transport mechanism linked to a Na+ pump and similar to the sodium-dependent glucose transporter.

Note

- M receptor activation →↓ CV function
- ↑ secretions and ↑ smooth muscle contraction
- All M receptor activators and blockers are nonspecific.

- Choline uptake is inhibited by **hemicholinium** (① in Figure II-2-1). ACh is synthesized from choline and acetyl-CoA via choline acetyltransferase (ChAT) and accumulates in synaptic vesicles.
- Presynaptic membrane depolarization opens voltage-dependent Ca^{2+} channels, and the influx of this ion causes fusion of the synaptic vesicle membranes with the presynaptic membrane, leading to exocytosis of ACh. **Botulinum toxin** (② in Figure II-2-1) interacts with synaptobrevin and other proteins to prevent ACh release and is used in blepharospasm, strabismus/hyperhidrosis, dystonia, and cosmetics.
- Some cholinergic nerve endings have presynaptic autoreceptors for ACh that on activation may elicit a negative feedback of transmitter release.
- Inactivation via acetylcholinesterase (AChE) is the major mechanism of termination of postjunctional actions of ACh.
- AChE is a target for inhibitory drugs (indirect-acting cholinomimetics). Note that such drugs can influence cholinergic function only at innervated sites where ACh is released.
- Reversible AChE inhibitors (③ in Figure II-2-1) include edrophonium, physostigmine, and neostigmine. Irreversible AChE inhibitors include malathion, and parathion.
- Postjunctional receptors (N and M) (④ in Figure II-2-1) activated by ACh are major targets for both activating drugs (direct-acting cholinomimetics) and blocking agents.

M Receptor Location and Function

High-Yield

Table II-2-1. Muscarinic Receptor Activation

Target		Receptor	Response
Eye	Sphincter	M_3	Contraction—miosis
	Ciliary muscle	M_3	Contraction—accommodation for near vision
Heart	SA node	M_2	↓ Heart rate (HR)—negative chronotropy
	AV node	M_2	↓ Conduction velocity—negative dromotropy
			No effects on ventricles, Purkinje system
Lungs	Bronchioles	M_3	Contraction—bronchospasm
	Glands	M_3	↑ Secretion
GI tract	Stomach	M_3	↑ Motility—cramps
	Glands	M_1	↑ Secretion
	Intestine	M_3	Contraction—diarrhea, involuntary defecation
Bladder		M_3	Contraction (detrusor), relaxation (trigone/sphincter), voiding, urinary incontinence
Sphincters		M_3	Relaxation, except lower esophageal, which contracts
Glands		M_3	↑ Secretion—sweat (thermoregulatory), salivation, and lacrimation
Blood vessels (endothelium)		M_3	Dilation (via NO/endothelium-derived relaxing factor)—no innervation, no effects of indirect agonists

Table II-2-2. Cholinergic Receptor Mechanisms

M_1 and M_3	G_q coupled	↑ phospholipase C →↑ IP_3, DAG, Ca^{2+}
M_2	G_i coupled	↓ adenylyl cyclase →↓ cAMP
N_N and N_M	No 2nd messengers	activation (opening) of Na/K channels

MUSCARINIC RECEPTOR ACTIVATORS

Muscarinic Agonists

Table II-2-3. Properties of Direct-Acting Cholinomimetics

Drug	Activity	AChE Hydrolysis	Clinical Uses
ACh	M and N	+++	Short half-life—no clinical use
Bethanechol	M	–	Rx—ileus (postop/neurogenic), urinary retention
Methacholine	M > N	+	Dx—bronchial hyperreactivity
Pilocarpine, cevimeline	M	–	Rx—xerostomia, glaucoma (pilocarpine)

Acetylcholinesterase Inhibitors

High-Yield

Table II-2-4. Properties of Indirect-Acting Cholinomimetics

Drug	Characteristics	Clinical Uses
Edrophonium	Short-acting	Dx—myasthenia gravis
Physostigmine	Tertiary amine (enters CNS)	Rx—glaucoma; antidote in atropine overdose
Neostigmine, pyridostigmine	Quaternary amines (no CNS entry)	Rx—ileus, urinary retention, myasthenia gravis, reversal of nondepolarizing NM blockers
Donepezil, rivastigmine	Lipid-soluble (CNS entry)	Rx—Alzheimer disease
Organophosphates	Lipid-soluble, irreversible inhibitors	Note: used as insecticides (malathion, parathion) and as nerve gas (sarin)

Clinical Correlate

Alzheimer disease is late-onset dementia with progressive memory loss and cognitive decline. Neuropathology includes neurofibrillary tangles, amyloid plaques, and loss of ACh neurons in the Meynert nucleus—a rationale for the clinical use of AChE inhibitors.

Toxicity of AChE Inhibitors

High-Yield

Excessive muscarinic and nicotinic stimulations

- Muscarinic effects:
  - Diarrhea
  - Urination
  - Miosis
  - Bradycardia
  - Bronchoconstriction
  - Lacrimation
  - Salivation
  - Sweating
  - CNS stimulation
- Nicotinic effects:
  - Skeletal muscle excitation followed by paralysis
  - CNS stimulation

Management

- Muscarinic effects: atropine
- Regeneration of AChE: pralidoxime (2-PAM)
- Time-dependent aging requires use of 2-PAM as soon as possible

Classic Clue

AChE inhibitor poisoning: "Dumbbeelss"

Diarrhea

Urination

Miosis

Bradycardia

Bronchoconstriction

Emesis

Excitation (CNS/muscle)

Lacrimation

Salivation

Sweating

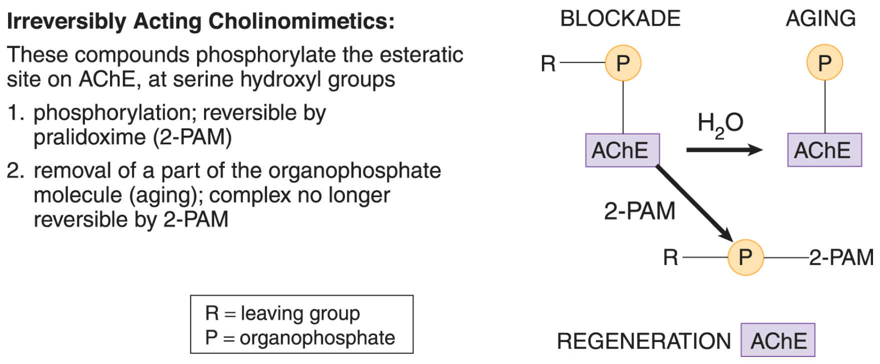


Figure II-2-2. Effects of Organophosphate on AChE

MUSCARINIC RECEPTOR ANTAGONISTS

Atropine

- Prototype of the class
- As a tertiary amine, it enters CNS
- Other M blockers differ mainly in their pharmacokinetic properties

Pharmacologic Effects

High-Yield

- Atropine effects in order of increasing dose:
  - Decreased secretions (salivary, bronchiolar, sweat)
  - Mydriasis and cycloplegia
  - Hyperthermia (with resulting vasodilation)
  - Tachycardia
  - Sedation
  - Urinary retention and constipation
  - Behavioral: excitation and hallucinations
- Other classes of drugs with antimuscarinic pharmacology:
  - Antihistamines
  - Tricyclic antidepressants
  - Antipsychotics
  - Quinidine
  - Amantadine
  - Meperidine
- Treatment of acute intoxication: symptomatic ± physostigmine

Table II-2-5. Clinical Uses and/or Characteristics of M Blockers

Drug	Clinical Uses and/or Characteristics
Atropine	Antispasmodic, antisecretory, management of AChE inhibitor OD, antidiarrheal, ophthalmology (but long action)
Tropicamide	Ophthalmology (topical)
Ipratropium, tiotropium	Asthma and COPD (inhalational)—no CNS entry, no change in mucus viscosity
Scopolamine	Used in motion sickness, causes sedation and short-term memory block
Benztropine, trihexyphenidyl	Lipid-soluble (CNS entry) used in parkinsonism and in acute extrapyramidal symptoms induced by antipsychotics
Oxybutynin	Used in overactive bladder (urge incontinence)

Recall Question

The action of botulinum toxin is through which of the following mechanisms?

A. Direct action on acetylcholine esterase

B. Direct action on muscarinic receptors

C. Prevention of acetylcholine release

D. Prevention of choline uptake

Answer: C

Bridge to Physiology

ANS Dominance

For effector tissues with dual innervation, PANS is dominant. These include the SA and AV nodes of the heart, the pupil, GI and GU muscles, and sphincters. SANS is dominant only in terms of vascular tone and thermoregulatory sweat glands.

NICOTINIC RECEPTOR ANTAGONISTS

Ganglion Blocking Agents

- Drugs: hexamethonium and mecamylamine
- Reduce the predominant autonomic tone
- Prevent baroreceptor reflex changes in heart rate

Table II-2-6. Nicotinic Receptor Activation

Target	Receptor	Response
Adrenal medulla	N_N	Secretion of epinephrine and NE
Autonomic ganglia	N_N	Stimulation—net effects depend on PANS/SANS innervation and dominance
Neuromuscular junction	N_M	Stimulation—twitch/hyperactivity of skeletal muscle

Note: N receptors desensitize very quickly upon excessive stimulation.

Table II-2-7. Effects of Ganglion Blocking Agents

Effector	System	Effect of Ganglion Blockade
Arterioles	SANS	Vasodilation, hypotension
Veins	SANS	Dilation, ↓ venous return, ↓ CO
Heart	PANS	Tachycardia
Iris	PANS	Mydriasis
Ciliary muscle	PANS	Cycloplegia
GI tract	PANS	↓ tone and motility—constipation
Bladder	PANS	Urinary retention
Salivary glands	PANS	Xerostomia
Sweat glands	SANS	Anhydrosis

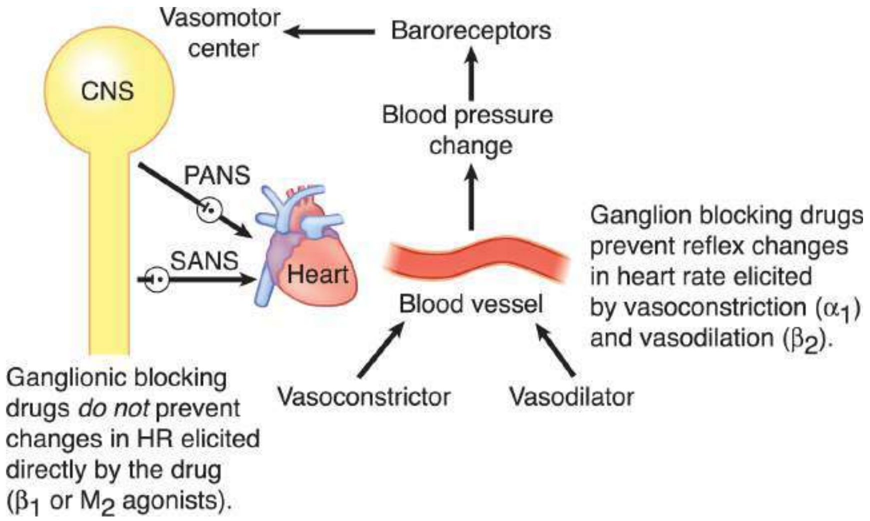


Figure II-2-3. Algorithm: Reflex Control of Heart Rate

Neuromuscular Blocking Drugs

See CNS Pharmacology, chapter on Drugs Used in Anesthesia.

Adrenergic Pharmacology 3

Learning Objectives

- ❑ Answer questions about catecholamine synthesis, action, and degradation
- ❑ Explain information related to direct-acting adrenoceptor agonists and indirect-acting adrenergic receptor agonists
- ❑ Differentiate between alpha receptor antagonists and beta receptor antagonists

ADRENERGIC NEUROEFFECTOR JUNCTIONS

Synthesis and Release of NE

High-Yield

The important aspects of the adrenergic neuroeffector junction are summarized below.

1 MAO inhibitors
2 Releasers
3 Reuptake blockers
4 α_2 agonists and antagonists
5 Agonists and blockers of α_1, β_1 receptors

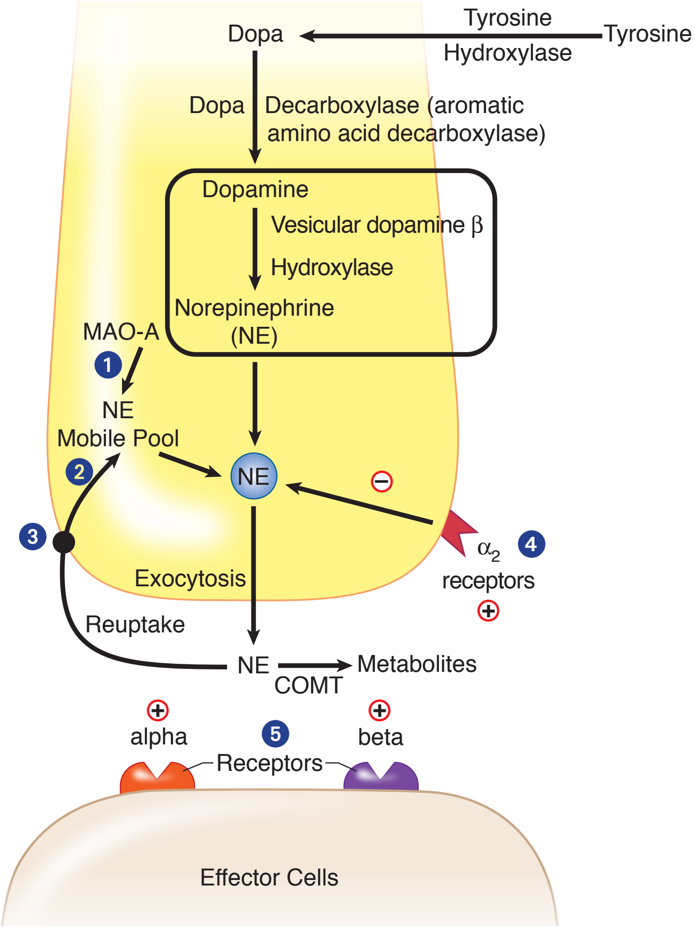


Figure II-3-1. Adrenergic Neuroeffector Junction

Tyrosine is actively transported into nerve endings and is converted to dihydroxyphenylalanine (DOPA) via tyrosine hydroxylase. This step is rate limiting in the synthesis of NE. DOPA is converted to dopamine (DA) via L-aromatic amino acid decarboxylase (DOPA decarboxylase). DA is taken up into storage vesicles where it is metabolized to NE via DA beta hydroxylase. Inactivation of NE via monoamine oxidase A (MAO-A) (1) may regulate prejunctional levels of transmitter in the mobile pool (2) but not the NE stored in granules.

Presynaptic membrane depolarization opens voltage-dependent Ca^{2+} channels. Influx of this ion causes fusion of the synaptic granular membranes, with the presynaptic membrane leading to NE exocytosis into the neuroeffector junction. NE then activates postjunctional receptors (5), leading to tissue-specific responses depending on the adrenoceptor subtype activated.

Termination of NE actions is mainly due to removal from the neuroeffector junction back into the sympathetic nerve ending via an NE reuptake transporter system (3). At some sympathetic nerve endings, the NE released may activate prejunctional alpha adrenoceptors (4) involved in feedback regulation, which results in decreased release of the neurotransmitter. Metabolism of NE is by catechol-O-methyltransferase (COMT) in the synapse or MAO_A in the prejunctional nerve terminal.

Adrenergic Receptor Location and Function

High-Yield

Table II-3-1. Adrenergic Receptor Activation

Receptor	Response
α_1	
Eye: radial (dilator) muscle	Contraction: mydriasis
Arterioles (skin, viscera)	Contraction: ↑ TPR, ↑ diastolic pressure, ↑ afterload
Veins	Contraction: ↑ venous return, ↑ preload
Bladder trigone and sphincter and prostatic urethra	Contraction: urinary retention
Male sex organs	Vas deferens: ejaculation
Liver	↑ glycogenolysis
Kidney	↓ renin release
α_2	
Prejunctional nerve terminals	↓ transmitter release and NE synthesis
Platelets	Aggregation
Pancreas	↓ insulin secretion
β_1	
Heart SA node	↑ HR (positive chronotropy)
AV node	↑ conduction velocity (positive dromotropy)
Atrial and ventricular muscle	↑ force of contraction (positive inotropy), conduction velocity, CO and oxygen consumption
His-Purkinje	↑ automaticity and conduction velocity
Kidney	↑ renin release
β_2 (mostly not innervated)	
Blood vessels (all)	Vasodilation: ↓ TPR: ↓ diastolic pressure, ↓ afterload
Uterus	Relaxation
Bronchioles	Dilation
Skeletal muscle	↑ glycogenolysis: contractility (tremor)
Liver	↑ glycogenolysis
Pancreas	↑ insulin secretion
D_1 (peripheral)	
Renal, mesenteric, coronary vasculature	Vasodilation: in kidney ↑ RBF, ↑ GFR, ↑ Na^+ secretion

Note

Adrenoceptor Sensitivity

Beta receptors are usually more sensitive to activators than alpha receptors. With drugs that exert both effects, the beta responses are dominant at low doses; at higher doses, the alpha responses will predominate.

Note

Dopamine Use in Shock

D_1 → β_1 → α_1

increasing doses of dopamine

Fenoldopam is a D_1 agonist used for severe hypertension.

Table II-3-2. Mechanisms Used by Adrenergic Receptors

α_1	G_q coupled	↑ phospholipase C →↑ IP_3, DAG, Ca^{2+}
α_2	G_i coupled	↓ adenylyl cyclase → ↓ cAMP
$\beta_1\beta_2$ D_1	G_s coupled	↑ adenylyl cyclase → ↑ cAMP

DIRECT-ACTING ADRENOCEPTOR AGONISTS

α_1 Agonists

High-Yield

Systemically, alpha-1 agonists increase mean BP via vasoconstriction.

- Increased BP may elicit a reflex bradycardia
- Cardiac output may be ↓ but also offset by ↑ venous return
- Drugs and uses:
  - **Phenylephrine:** nasal decongestant and ophthalmologic use (mydriasis without cycloplegia), hypotensive states

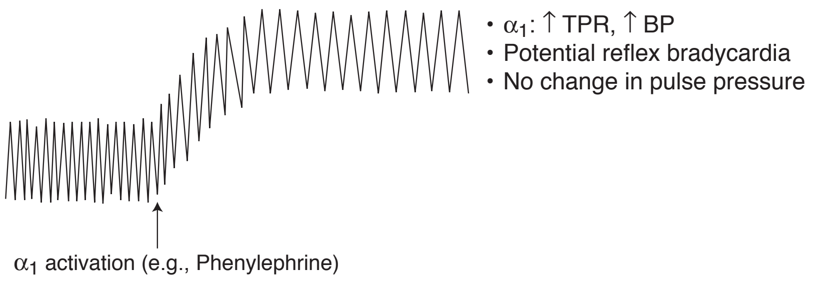


Figure II-3-2. Effect of Alpha Activators on Heart Rate and Blood Pressure

α_2 Agonists

Alpha-2 agonists stimulate prejunctional receptors in the CNS to decrease sympathetic outflow. Their primary use is for mild to moderate HTN.

- Drugs and uses: **clonidine** and **methyldopa** (mild to moderate hypertension)
- *See* Cardiovascular section.

β Agonists

Systemically, beta-agonists decrease mean BP via vasodilation (β_2) and and increase HR (β_1).

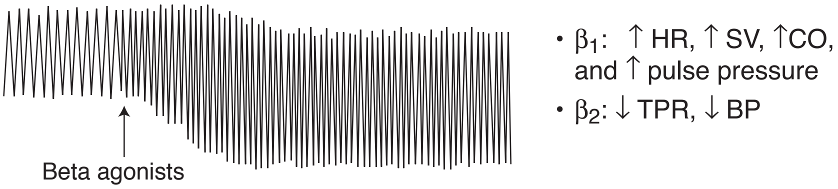


Figure II-3-3. Effect of Beta Receptor Activation on Heart Rate and Blood Pressure

- Drugs and uses:
  - Isoproterenol ($\beta_1 = \beta_2$)
  - Dobutamine ($\beta_1 > \beta_2$): congestive heart failure
  - Selective β_2 agonists: salmeterol, albuterol, terbutaline (asthma); terbutaline (premature labor)

Mixed-Acting Agonists: Norepinephrine vs. Epinephrine

High-Yield

Norepinephrine (α_1, α_2, β_1)

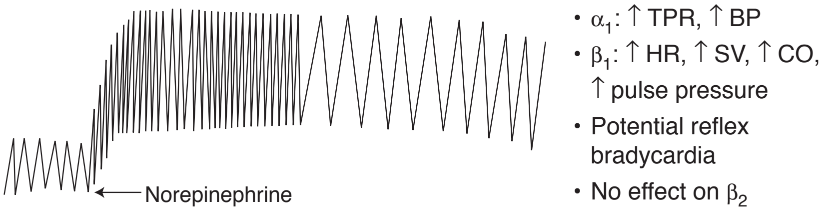


Figure II-3-4. Effect of Norepinephrine on Heart Rate and Blood Pressure

Epinephrine (α_1, α_2, β_1, β_2)

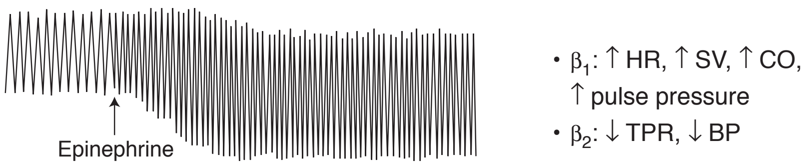


Figure II-3-5a. Effect of Low-dose Epinephrine on Heart Rate and Blood Pressure

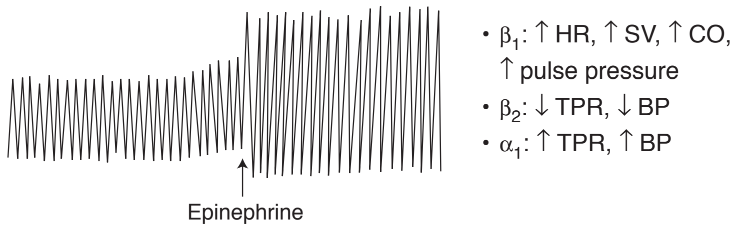


Figure II-3-5b. Effect of Medium-Dose Epinephrine on Heart Rate and Blood Pressure

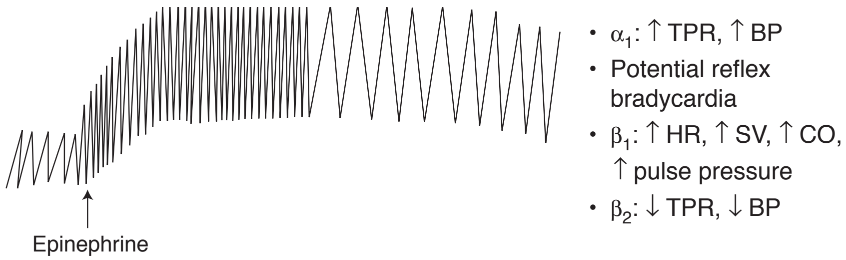


Figure II-3-5c. Effect of High-dose Epinephrine Is Similar to Norepinephrine

- Dose-dependent effects:
  - Low-dose: β_1, β_2 stimulation (*see* Figure II-3-5a)
  - High-dose: α_1, β_1 (β_2) (*see* Figure II-3-5c)
- β_2-specific effects:
  - Smooth muscle relaxation: bronchioles, uterus, blood vessels
  - Metabolic effects:
    - ↑ glycogenolysis (muscle and liver)
    - ↑ gluconeogenesis
    - ↑ mobilization and use of fat

- Differentiation of high-dose epinephrine versus norepinephrine:
  - Epinephrine reversal: Use of α_1 blocker to reverse hypertension to hypotension in a patient receiving too much epinephrine
  - Hypertension was due to predominant α_1 tone on the vasculature
  - Hypotension results from unmasking β_2 receptors

Uses of Norepinephrine and Epinephrine

- Cardiac arrest
- Adjunct to local anesthetic
- Hypotension
- Anaphylaxis (epinephrine only)
- Asthma (epinephrine only)

INDIRECT-ACTING ADRENERGIC RECEPTOR AGONISTS

Releasers

Releasers displace norepinephrine from the mobile pool.

- Drug interaction: MAO_A inhibitors (hypertensive crisis)
- **Tyramine** (red wine, cheese)
  - Oral bioavailability is limited by MAO-A metabolism in gut and liver
  - MAO-A inhibition ↑ bioavailability, resulting in hypertensive crisis
- **Amphetamines**
  - Clinical use of methylphenidate in narcolepsy and ADHD
  - Psychostimulant due to central release of DA, NE, 5HT
- **Ephedrine** (cold medication)

Reuptake Inhibitors

- Cocaine
- Tricyclic antidepressant (in part)

ADRENERGIC ANTAGONISTS

α Receptor Antagonists

Alpha-receptor antagonists decrease TPR and decrease mean BP.

- May cause reflex tachycardia and salt and water retention
- Major uses:
  - Hypertension
  - Pheochromocytoma (nonselective α blocker)
  - Benign prostatic hyperplasia (BPH; selective α_1 blocker)

Clinical Correlate

- Indirect-acting adrenoceptor agonists act only on effector tissues innervated by SANS.
- Denervated effector tissues are nonresponsive because these drugs act either to release transmitter from nerve terminals or to inhibit neurotransmitter reuptake.

Note

Forms of MAO

- MAO type A: mainly in liver, but Anywhere (metabolizes NE, 5HT, and tyramine)
- MAO type B: mainly in Brain (metabolizes DA)

Clinical Correlate

Chronic use of beta blockers (e.g., in angina, HTN) leads to receptor upregulation.

During withdrawal from use, it is important to taper dose to avoid excessive cardiovascular effects (rebound effects) of endogenous amines.

Clinical Correlate

Glucagon and the Heart

Positive inotropic and chronotropic, not via activation of β_1 receptors, but through glucagon receptors that are G-protein linked to adenylyl cyclase → basis for its use in beta-blocker overdose.

- Drugs:
  - Nonselective blocker: phentolamine (competitive inhibitor), phenoxybenzamine (noncompetitive inhibitor)
  - Selective α_1 blocker: prazosin, doxazosin, terazosin, tamsulosin
  - Selective α_2 blocker: mirtazapine (used as antidepressant)

β Receptor Antagonists

High-Yield

- β_1 blockade:
  - ↓ HR, ↓ SV, ↓ CO
  - ↓ renin release
- β_2 blockade:
  - May precipitate bronchospasm (in asthmatics) and vasospasm (in patients with vasospastic disorders)
  - ↓ aqueous humor production
  - Metabolic effects
    - Blocks glycogenolysis, gluconeogenesis
    - ↑ LDLs, TGs

Table II-3-3. Characteristics of Some Beta Blockers

Drugs	β1-Selective	ISA	Sedation	Blood Lipids
Acebutolol	+	++	+	–
Atenolol	+	–	–	↑↑
Metoprolol	+	–	+	↑↑
Pindolol	–	++	+	–
Propranolol	–	–	+++	↑↑
Timolol	–	–	++	↑↑

- Cardioselectivity (β_1):
  - Less effect on vasculature, bronchioles, uterus, and metabolism
  - Safer in asthma, diabetes, peripheral vascular diseases
- Intrinsic sympathomimetic activity (ISA):
  - Act as partial agonists
  - Less bradycardia (β_1)
  - Slight vasodilation or bronchodilation (β_2)
  - Minimal change in plasma lipids (β_2)

- Pharmacokinetic properties: no CNS entry of atenolol
- General uses of beta-blockers:
  - Angina, hypertension, post-MI (all drugs)
  - Antiarrhythmics (class II: propranolol, acebutolol, esmolol)
  - Glaucoma (timolol)
  - Migraine, thyrotoxicosis, performance anxiety, essential tremor (propranolol)
- Combined alpha-1 and beta blocking activity:
  - Labetalol and carvedilol
  - Use in CHF (carvedilol) and in hypertensive emergencies (labetalol)
- K^+-channel blockade and β-blocking activity: sotalol

Recall Question

Which of the following directly results from activation of the beta 2 receptor?

A. Decrease in blood pressure

B. Increase in cardiac output

C. Increase in heart rate

D. Increase in stroke volume

Answer: A

Autonomic Drugs: Glaucoma Treatment and ANS Practice Problems

4

Learning Objectives

- ❏ Solve problems concerning glaucoma treatment

GLAUCOMA TREATMENT

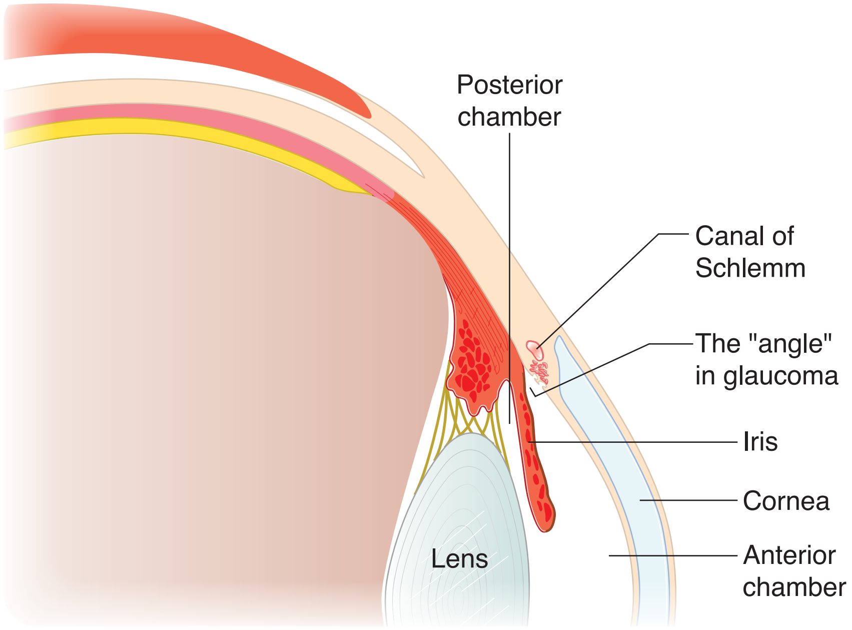


Figure II-4-1. Anatomy of the Eye Showing Irido-Corneal Angle Where Aqueous Humor Is Recirculated

Open-Angle Glaucoma

Open-angle glaucoma is a chronic condition with increased intraocular pressure (IOP) due to decreased reabsorption of aqueous humor. It leads to progressive (painless) visual loss and, if left untreated, blindness. IOP is a balance between fluid formation and its drainage from the globe.

Strategies in drug treatment of glaucoma include the use of beta blockers to decrease formation of fluid by ciliary epithelial cells and the use of muscarinic activators to improve drainage through the canal of Schlemm.

Note

Antimuscarinic drugs and α_1 agonists are contraindicated in closed-angle glaucoma.

Closed-Angle Glaucoma

Closed-angle glaucoma is an acute (painful) or chronic (genetic) condition with increased IOP due to blockade of the canal of Schlemm.

Emergency drug management prior to surgery usually involves cholinomimetics, carbonic anhydrase inhibitors, and/or mannitol.

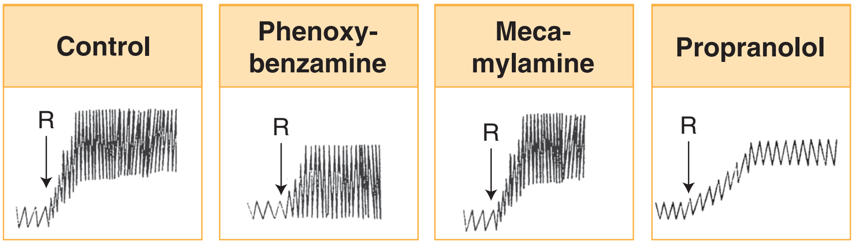
Treatment

Table II-4-1. Mechanism of Action of Drugs Used to Treat Glaucoma

Drug	Drug Class	Mechanism of Action
Pilocarpine	Cholinomimetic	Activation of M receptors causes contraction of ciliary muscle, which increases flow through the canal of Schlemm
Timolol	Beta blockers	Block actions of NE at ciliary epithelium ↓ aqueous humor formation

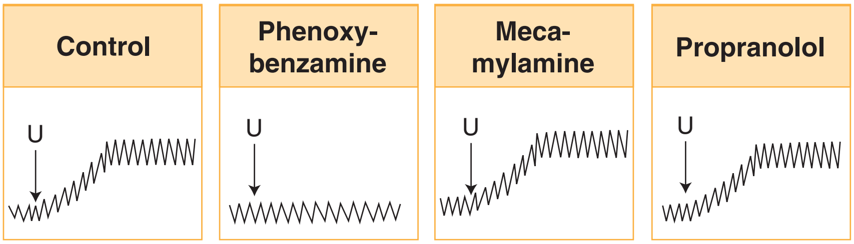
ANS PRACTICE PROBLEMS

1.



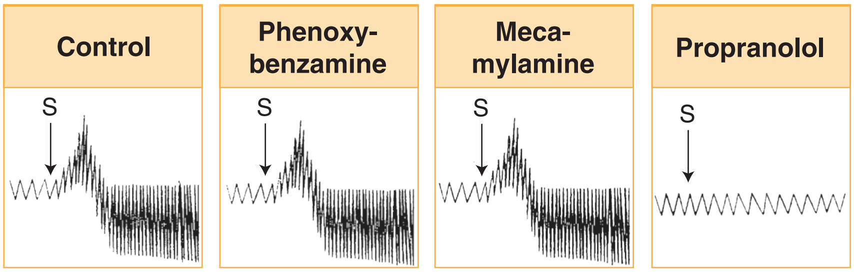
R is A. Epinephrine
B. Norepinephrine
C. Phenylephrine
D. Isoproterenol
E. Terbutaline

2.



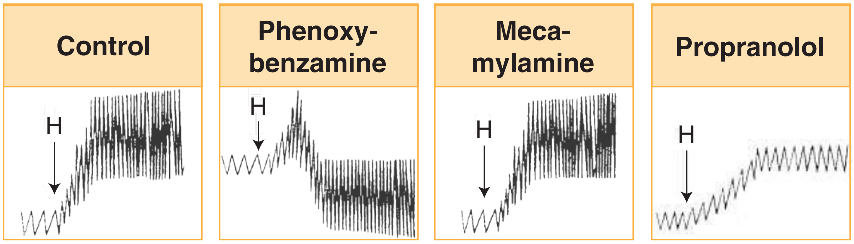
U is A. Epinephrine
B. Norepinephrine
C. Phenylephrine
D. Isoproterenol
E. Tyramine

3.



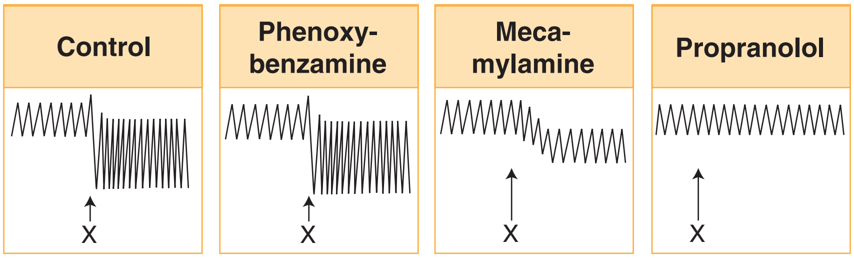
S is
A. Epinephrine
B. Norepinephrine
C. Phenylephrine
D. Isoproterenol
E. Terbutaline

4.



H is
A. Epinephrine
B. Norepinephrine
C. Phenylephrine
D. Isoproterenol
E. Albuterol

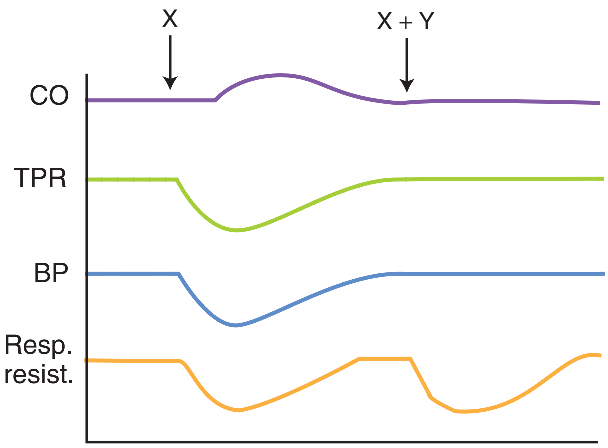
5.



Drug X is most like

A. Epinephrine
B. Isoproterenol
C. Norepinephrine
D. Phenylephrine
E. Terbutaline

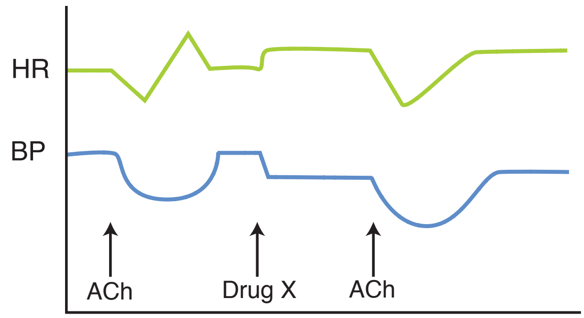
6.



X and Y are, respectively:

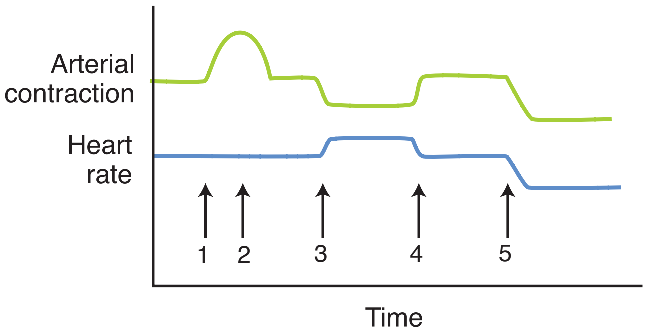
A. Isoproterenol and Propranolol
B. Epinephrine and Phenoxybenzamine
C. Norepinephrine and Phentolamine
D. Terbutaline and Phenylephrine
E. Acetylcholine and Hexamethonium

7.



What is drug X?
A. Hexamethonium
B. Neostigmine
C. Atropine
D. Scopolamine
E. Ipratropium

8.



Given the following information:

- Contractile force is measured in an isolated arterial preparation, and heart rate is measured in an isolated heart preparation.
- One drug is added at each specified time.
- No washout between drugs

A. Bethanechol

B. Epinephrine

C. Phenoxybenzamine

D. Pindolol

E. Phenylephrine

Time 1:

Time 2:

Time 3:

Time 4:

Time 5:

9. The circles below represent the size of the pupils of a patient's eyes, without treatment and with two different treatments.

The responses are compatible with the conclusion that the left eye had:

A. been pretreated with atropine.

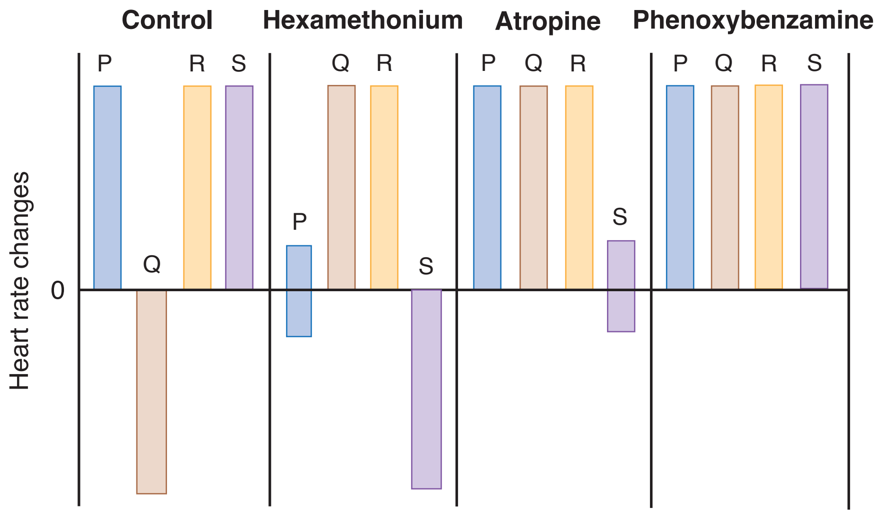
B. been pretreated with prazosin.

C. been pretreated with propranolol.

D. been pretreated with physostigmine.

E. denervation of the radial muscle.

10.



A.	Acetylcholine	
B.	Hydralazine	P is
C.	Norepinephrine	Q is
D.	Isoproterenol	R is
E.	Edrophonium	S is

ANSWERS AND EXPLANATIONS

1. **Answer: B.** The effects of Drug R are changed by treatment with either an alpha or beta-blocker, so Drug R must have activity at both receptors (**choices C**, **D**, and **E** are ruled out). A pressor dose of epinephrine would be "reversed" by an alpha-blocker, not just decreased! Drug R is norepinephrine.

2. **Answer: C.** The effects of Drug U are changed by treatment with the alpha-blocker, but not by the beta-blocker. Drug U must be an alpha-activator with no beta actions—the only choice is phenylephrine.

3. **Answer: D.** The effects of Drug S are changed by treatment with the beta-blocker, but not by the alpha blocker (**choices A**, **B**, and **C** are ruled out). Terbutaline is β_2 selective and would not increase heart rate directly. Drug S is isoproterenol. Note that option A would have been a possibility but one would have to assume a low-dose of epinephrine.

4. **Answer: A.** The effects of Drug H are changed by treatment with either an alphaor beta- blocker, so Drug H must have activity at both receptors (**choices C**, **D**, and **E** are ruled out). "Reversal" of a pressor effect can only occur if the drug has β_2 activity (**choice B** is ruled out). Drug H is epinephrine.

5. **Answer: E.** Mecamylamine blocked reflexed tachycardia induced by Drug X, which dropped blood pressure by vasodilation. Propranolol prevented all responses. Drug X is a β_2 agonist (terbutaline).

6. **Answer: D.** Drug X decreases TPR and BP, eliciting a reflex sympathetic discharge (note delay in response), resulting in increased CO. There is no direct effect on CO (**choices A**, **B**, **C**, and **E** are ruled out). Drugs X and Y are terbutaline and phenylephrine. Note that the alpha agonist does not antagonize the decrease in respiratory resistance (a β_2 response).

7. **Answer: A.** ACh (used as a drug) decreases blood pressure and heart rate, but the latter effect is overcome and reversed by a sympathetic reflex. Because Drug X abolishes only the reflex tachycardia, it must be the ganglion blocker hexamethonium (**choice A**). Remember, AChE inhibitors do not vasodilate because there is no parasympathetic innervation of the vasculature!

8. No autonomic reflexes are possible in isolated preparations, so every action observed in this experiment is due to a direct action of the drug. **Time 1:** Arterial contraction is due to an alpha-1 agonist (**choice E**). **Time 2:** Arterial contraction is reversed by an alpha antagonist (**choice C**). **Time 3:** Tachycardia and arterial dilation are due to beta-1 and beta-2 actions (**choice B** at a low dose). **Time 4:** Tachycardia and arterial dilation are reversed by a non-selective beta blocker (**choice D**). **Time 5:** Bethanechol (**choice A**) causes both arterial relaxation and bradycardia.

9. **Answer: D.** Classic example showing that denervated tissues do not respond to indirect-acting agonists. In this case, amphetamine fails to cause mydriasis in the left eye, but this eye is more responsive than the right eye to phenylephrine (denervation supersensitivity).

10. Block of tachycardia due to Drug P by hexamethonium is indicative of a sympathetic reflex that follows a decrease in BP due to a vasodilator (**choice B**). "Reversal" of bradycardia due to Drug Q by hexamethonium indicates a vagal reflex elicited by vasoconstriction (e.g., alpha activation) masking cardiac stimulation (e.g., beta activation) typical of norepinephrine (**choice C**). Tachycardia due to Drug R is unaffected by any antagonist, indicative of a beta activator (**choice D**). "Reversal" of tachycardia due to Drug S by hexamethonium indicates a sympathetic reflex masking a vagomimetic action typical of a muscarinic activator (**choice A**); this is confirmed by the effect of atropine

Autonomic Drug List and Practice Questions

5

Cholinergic Receptor Activators

Direct activators: bethanechol (M), methacholine (M and N), nicotine (N), pilocarpine (M), cevimeline (M)

AChE inhibitors: reversible—edrophonium, physostigmine, neostigmine, pyridostigmine, donepezil, rivastigmine

AChE inhibitors: irreversible—malathion, parathion

Cholinergic Receptor Antagonists

Muscarinic blockers: atropine, benztropine, ipratropium, scopolamine

Ganglionic blockers: hexamethonium, mecamylamine

Adrenergic Receptor Activators

α_1 agonists: phenylephrine

α_2 agonists: clonidine, methyldopa

β agonists: isoproterenol, ($\beta_1 = \beta_2$), dobutamine ($\beta_1 > \beta_2$)

β_2 agonists: albuterol, terbutaline, salmeterol

Mixed: dopamine (D_1, β_1, α_1), epinephrine (α_1, α_2, β_1, β_2), norepinephrine (α_1, α_2, β_1)

Indirect-acting: amphetamine, cocaine, ephedrine, tyramine

Adrenergic Receptor Antagonists

$\alpha_1$antagonists: doxazosin, prazosin, terazosin

α_2 antagonists: mirtazapine

Mixed α antagonists: phenoxybenzamine, phentolamine

β_1 (cardioselective) antagonists: acebutolol, atenolol, metoprolol

β_1, β_2 (nonselective): pindolol, propranolol, timolol

α_1 and β antagonists: carvedilol, labetalol

PRACTICE QUESTIONS

1. Alpha-1 agonists cause reflex bradycardia, which can be blocked by

   A. atenolol
   B. atropine
   C. mirtazapine
   D. phenylephrine
   E. propranolol

2. Which one of the following effects is caused by the ingestion of mushrooms that contain pilocarpine?

   A. Tachycardia
   B. Bronchodilation
   C. Diarrhea
   D. Hypertension
   E. Hyperthermia

3. Increasing the concentration of norepinephrine in adrenergic synapses leads to

   A. activation of dopa decarboxylase
   B. increased release of norepinephrine
   C. activation of presynaptic G_i coupled receptors
   D. stimulation of MAO
   E. activation of tyrosine hydroxylase

4. Urination in the human subject is decreased by

   A. muscarinic agonists
   B. muscarinic antagonists
   C. AChase inhibitors
   D. Nicotinic agonists
   E. Spider venom

5. A 5-year-old child becomes ill while visiting relatives who have a farm in Arkansas. His symptoms include severe abdominal cramps with vomiting and diarrhea and profuse lacrimation and salivation. Pupillary constriction is marked. The most likely cause is exposure to

   A. herbicides
   B. antifreeze
   C. lead-based paint
   D. insecticides
   E. rat poison

6. The activation of muscarinic receptors in bronchiolar smooth muscle is associated with

   A. activation of adenylyl cyclase
   B. decrease in cAMP formation mediated by G-proteins
   C. increase in IP_3 and DAG
   D. inhibition of protein kinase C
   E. opening of Na^+/K^+ cation channels

7. Ganglion blocking agents are of little clinical value today but they are important drugs to know for solving cardiovascular drug problems because they can block

   A. all muscarinic receptors
   B. all nicotinic receptors
   C. all autonomic reflexes
   D. the direct actions of drugs on blood vessels
   E. the direct actions of drugs on the heart

8. An 11-year-old boy was brought to the ER by some of his friends because he “started going crazy” after eating seeds from a plant while “trying to get high.” The boy was incoherent; his skin was hot and dry. His pupils were dilated and unresponsive to light. Blood pressure was 180/105 mm Hg, pulse 150/min, and rectal temp 40 C (104 F). The presumptive diagnosis was drug toxicity due to the ingestion of a compound similar to

   A. cannabis
   B. digoxin
   C. mescaline
   D. phencyclidine
   E. scopolamine

9. Reflex tachycardia caused by the systemic administration of albuterol can be blocked by what drug?

   A. dobutamine
   B. prazosin
   C. phenylephrine
   D. metoprolol
   E. low-dose epinephrine

10. Cardiovascular effects of a new drug (X) that activates autonomic receptors are shown in the table below:

Parameter	Control	Drug X
Systolic BP	120 mm Hg	110 mm Hg
Diastolic BP	85 mm Hg	55 mm Hg
Heart rate	60/min	120/min

The most probable receptor affinities of drug X are

A. α_1, α_2
B. α_1, α_2, β_1
C. β_1, β_2
D. M_2
E. N_M

11. Thermoregulatory sweat glands in the body utilize what type of pathway?

A. Cholinergic nerves and muscarinic receptors
B. Adrenergic nerves and alpha-1 receptors
C. Adrenergic nerves and beta-2 receptors
D. Cholinergic nerves and N_M receptors
E. Neurohumorally-released epinephrine

12. Activation of postsynaptic M_2 receptors on the heart is associated with

    A. activation of adenylyl cyclase
    B. decrease in cAMP formation
    C. increase in IP_3 and DAG
    D. inhibition of protein kinase C
    E. opening of Na^+/K^+ cation channels

13. The data in the table below show the effects of four drugs (#1–4) on mean blood pressure administered as individual agents before and after treatment with prazosin. The arrows denote the direction and intensity of drug actions on blood pressure.

Condition	Drug #1	Drug #2	Drug #3	Drug #4
Before prazosin	↑↑	↑↑	↓↓	↑
After prazosin	↑	↑	↓↓	↓

    The order of drug #1 through drug #4 is best represented by

    A. epinephrine—tyramine—isoproterenol—norepinephrine
    B. tyramine—isoproterenol—norepinephrine—epinephrine
    C. norepinephrine—isoproterenol—epinephrine—tyramine
    D. isoproterenol—epinephrine—tyramine—norepinephrine
    E. norepinephrine—tyramine—isoproterenol—epinephrine

14. Prior to an eye exam a patient is given a drug that causes mydriasis but has no effect on accommodation. What is the most likely identity of this drug?

    A. mecamylamine
    B. neostigmine
    C. pilocarpine
    D. phenylephrine
    E. tropicamide

15. Following a myocardial infarct, a 40-year-old man is being treated prophylactically with propranolol. You would be concerned about the use of this drug if the patient also had what comorbid condition?

    A. Essential tremor
    B. Glaucoma
    C. Classic/stable angina
    D. Supraventricular tachycardia
    E. Diabetes

16. Following pretreatment with a muscarinic receptor blocking agent, the IV administration of norepinephrine is likely to result in

   A. ↑HR and ↑ BP
   B. ↑ HR and ↓ BP
   C. ↓ HR and ↓ BP
   D. ↓ HR and ↑ BP
   E. no effect on HR, but ↑ BP

17. A 45-year-old man has recently been the recipient of a heart transplant. Which one of the following drugs is least likely to cause tachycardia in this patient?

   A. Amphetamine
   B. Dobutamine
   C. Epinephrine
   D. Isoproterenol
   E. Norepinephrine

18. A colleague with myasthenia gravis wants you to assist him to the ER because he is experiencing muscle weakness and has found it difficult to titrate his drug dosage because he has had the "flu." You note that he has a slight temperature, shallow respirations, and a gray-blue skin pallor. What would be the most appropriate drug to give to your colleague at this time?

   A. Albuterol
   B. Edrophonium
   C. Propranolol
   D. Physostigmine
   E. Scopolamine

19. Carvedilol is an effective antihypertensive agent that, like propranolol, is capable of blocking beta receptors. An important difference between the two drugs is that carvedilol

   A. is a selective blocker of cardiac β_1 receptors
   B. has intrinsic sympathomimetic activity
   C. is available only as eye drops
   D. has α_1 receptor blocking actions
   E. stimulates β_2 receptors in bronchioles

20. Neostigmine differs from pilocarpine in having effects on

   A. bladder tone
   B. bowel motility
   C. heart rate
   D. salivary glands
   E. skeletal muscle

Questions 21–23

The table below shows the effects of three receptor activators on heart rate in anesthetized animals, administered as individual drugs and following pretreatment with one of four different receptor antagonists. The arrows denote the direction of effects on heart rate; the symbol (–) denotes no change from normal HR.

Antagonist Pretreatment	Agonist 1	Agonist 2	Agonist 3
None	↑	↓	↓
Atropine	↑	–	↑
Prazosin	↑	–	↑
Propranolol	–	↓	↓
Mecamylamine	↑	–	↑

Identify the agonist drugs from the following list:

A. Acetylcholine
B. Low-dose epinephrine
C. Norepinephrine
D. Phenylephrine
E. Physostigmine

21. Agonist 1

22. Agonist 2

23. Agonist 3

ANSWERS AND EXPLANATIONS

1. **Answer: B.** Bradycardia due to vagal stimulation is elicited by activation of muscarinic receptors in the heart. Atropine, which is an antagonist at M receptors, blocks bradycardia elicited by stimulation of the vagus, including reflex bradycardia due to increases in mean BP caused by vasoconstrictors.

2. **Answer: C.** Pilocarpine is present in several mushroom species including *Amanita muscaria*, the ingestion of which is associated with the stimulation of M receptors (parasympathomimetic effects). Activation of muscarinic receptors in the GI tract causes diarrhea. The activation by pilocarpine of M receptors present on vascular endothelial cells would lead to hypotension (not hypertension) via the release of NO. All of the other effects listed are typical of muscarinic antagonists.

3. **Answer: C.** In sympathetic nerve endings presynaptic α_2 receptors are coupled to inhibitory G-proteins. These receptors serve an autoregulatory function to inhibit further neurotransmitter release and also to decrease the synthesis of norepinephrine.

4. **Answer: B.** Urinary retention is a well known adverse effect of drugs that have antagonist effects on muscarinic receptors. In addition to the prototypic drug atropine, M blockers include drugs used in Parkinson disease, such as benztropine. Acetylcholine directly and AChE inhibitors (edrophonium, physostigmine) indirectly activate M receptors in the GU system, causing bladder contraction with voiding and incontinence. Activation of nicotinic receptors in ANS ganglia would lead to the stimulation of PANS functions.

5. **Answer: D.** The symptoms of cholinergic excess seen in this child are indicative of exposure to insecticides such as the organophosphate parathion, which cause irreversible inhibition of acetylcholinesterase. Other symptoms may include CNS excitation and stimulation of the skeletal NMJ, ultimately leading to paralysis of respiratory muscles—"DUMBBEELSS." In addition to symptomatic support, management of AChE inhibitor poisoning involves the use of atropine and 2-PAM.

6. **Answer: C.** Muscarinic receptors present in bronchiolar smooth muscle are of the M_3 subtype coupled via G_q proteins to phospholipase C. Activation of this enzyme causes hydrolysis of phosphatidylinositol bisphosphate, with release of IP_3 and DAG (the latter activates protein kinase C). Decreased formation of cAMP mediated via a G_i protein occurs with activation of M_2 receptors such as those in the heart. Cation channel opening occurs in response to activation of nicotinic receptors.

7. **Answer: C.** Ganglion blockers (hexamethonium, mecamylamine) block N_N receptors at autonomic ganglia and the adrenal medulla. As such, they can block all autonomic reflexes including those elicited by changes in blood pressure. They have no effect on nicotinic receptors at the neuromuscular junction (N_M) or on the direct actions of drugs on the blood vessels or heart.

8. **Answer: E.** The signs and symptoms experienced by this boy are highly suggestive of the ingestion of a compound with strong muscarinic receptor-blocking actions. The leaves and seeds of jimsonweed (*Datura stramonium*) contain anticholinergic compounds, including atropine, hyoscyamine, and scopolamine—approximately 50 seeds may cause severe toxicity. In addition to symptomatic support, management of poisoning (or drug overdose) due to M blockers may involve use of the AChE inhibitor physostigmine.

9. **Answer: D.** Although used primarily via inhalation for asthma, systemic effects of albuterol include vasodilation due to its β_2 receptor activation. This can result in a decrease in TPR and mean BP, which elicits a reflex tachycardia. Reflex tachycardia could be blocked at the heart with a beta blocker such as metoprolol or by ganglion blockers (mecamylamine) which prevent all autonomic reflexes. Dobutamine stimulates beta-1 receptors causing tachycardia. Phenylephrine stimulates alpha-1 receptors which would raise TPR and BP and evoke a reflex bradycardia that doesn't block tachycardia caused by albuterol. Prazosin blocks alpha-1 receptors decreasing TPR and BP and causing a reflex tachycardia. Low-dose epinephrine stimulates beta-1 and beta-2 receptors and will cause tachycardia.

10. **Answer: C.** A decrease in mean blood pressure, an increase in pulse pressure, plus a marked increase in heart rate are characteristic of a drug such as isoproterenol. PVR and mean BP are decreased because of activation of β_2 receptors in the vasculature. Systolic BP decreases less than diastolic BP because of activation of β_1 receptors in the heart, leading to an increase in stroke volume, as well as the increase in heart rate.

11. **Answer: A.** Thermoregulatory sweat glands are innervated only by the sympathetic nervous system. The pathway is unusual in that the postganglionic neuron releases acetylcholine. Thus, the receptors on sweat glands are muscarinic (M_3). The term *neurohumoral* means "nerve-blood." The only site in the ANS where neurohumoral transmission occurs is the adrenal medulla, where sympathetic nerve activity elicits the release of catecholamines (mostly epinephrine) into the blood. Epinephrine cannot bind to muscarinic receptors.

12. **Answer: B.** Postsynaptic muscarinic receptors on the heart (M_2) are G_i protein coupled to inhibition of adenylyl cyclase and decreased formation of cAMP.

13. **Answer: E.** Of the drugs listed, only isoproterenol causes a decrease in mean blood pressure, because it activates beta receptors and has no effect on alpha receptors. This permits identification of drug #3 as isoproterenol. Prazosin is an alpha blocker, so one can anticipate that this drug would antagonize any increases in blood pressure that result from activation of α_1 receptors in the vasculature. Epinephrine (high dose), norepinephrine, and tyramine all exert pressor effects via activation of α_1 receptors. However, only epinephrine is active on β_2 receptors, and this action would be revealed by vasodilation and a reversal of its pressor effects following treatment with an alpha blocker—"epinephrine reversal." Thus, drug #4 can be identified as epinephrine.

14. **Answer: D.** Mydriasis can be caused by either a muscarinic antagonist or an alpha-1 agonist. Cycloplegia (paralysis of accommodation) is caused by a muscarinic antagonist, but accommodation is unaffected by an alpha-1 agonist such as phenylephrine. Remember accommodation is a parasympathetic function only. Ganglionic blockade with mecamylamine would cause mydriasis and cycloplegia similar to a muscarinic blocker.

15. **Answer: E.** Propranolol is a nonselective beta blocker that causes hypoglycemia by blocking glycogenolysis and gluconeogeneis in the liver and skeletal muscle. This is of particular concern in a patient with diabetes. The other conditions listed are all potential uses for beta blockers, including essential tremor where it is important to use a nonselective beta blocker.

16. **Answer: A.** Norepinephrine activates α_1 and β_1 receptors, causing increases in PVR and CO. The increase in mean BP can elicit reflex bradycardia (vagal outflow leads to stimulation of cardiac M receptors), which may overcome the direct stimulatory effects of NE on the heart. However, reflex bradycardia is not possible following pretreatment with an M blocker. Thus, HR increases because of the direct activation of cardiac β_1 receptors by NE.

17. **Answer: A.** This question is to remind you that indirect-acting sympathomimetics require innervation of the effector organ to exert effects. In this case, amphetamine would not be effective because the transplanted heart lacks sympathetic innervation; thus, there is no "mobile pool" of NE capable of being released by a drug. However, transplanted hearts retain receptors, including those (β_1) responsive to direct-acting sympathomimetics. Heart transplants are not responsive to AChE inhibitors because they, too, are indirect acting and require vagal innervation to exert effects on the heart.

18. **Answer: B.** Edrophonium is a very short-acting (reversible) AChE inhibitor that has been used in the diagnosis of myasthenia gravis. The drug is useful for distinguishing between muscle weakness attributable to excessive cholinergic receptor stimulation (usually due to overdose of a AChE inhibitor) and the symptoms of myasthenia (reflecting inadequate treatment). If symptoms improve with a single dose of edrophonium, then an increase in the dose of neostigmine or pyridostigmine is indicated. If symptoms worsen, then the dose of neostigmine should be reduced.

19. **Answer: D.** The effectiveness of carvedilol in the management of hypertension and in congestive heart failure appears to be due to a combination of antagonistic actions at both alpha and beta adrenoceptors. Carvedilol is not a β_1 selective blocking agent (unlike atenolol and metoprolol), and (unlike pindolol and acebutolol) it lacks intrinsic sympathomimetic activity.

20. **Answer: E.** As an inhibitor of AChE, neostigmine exerts effects to enhance the actions of ACh at all innervated effector sites where ACh is a neurotransmitter. These include all ANS ganglia, PANS postganglionic neuroeffector junctions, and SANS innervation of thermoregulatory sweat glands. Pilocarpine activates M receptors and has no effects at conventional dose levels on nicotinic receptors such as those in ANS ganglia and the skeletal NMJ.

21. **Answer: B.**

22. **Answer: D.**

23. **Answer: C.** Agonist 1 increases HR, presumably through direct activation of cardiac β_1 receptors because the effect is blocked by propranolol but is not influenced by the alpha blocker (prazosin), the ganglion blocker (mecamylamine), or blockade of M receptors (atropine). Only two of the listed drugs directly activate cardiac receptors: epinephrine and norepinephrine. For NE, any direct cardiac stimulation is counteracted by reflex bradycardia resulting from the increase in mean BP via its activation of α_1 receptors in blood vessels (it has no effects on β_2 vascular receptors). Therefore, agonist 1 is identified as epinephrine which activates both β_1 and β_2 receptors directly at low doses.

    To identify agonists 2 and 3, recognize that although the alpha blocker prazosin simply neutralizes the effect of agonist 2 on HR, it reverses the effect of agonist 3. This could occur only if agonist 3 was capable of β_1 receptor activation in the heart. Direct cardiac stimulation could occur with norepinephrine (agonist 3) but not with phenylephrine (agonist 2), which is a selective alpha-1 agonist.

PART III

Cardiac and Renal Pharmacology

Diuretics 1

Learning Objectives

❑ Answer questions about osmotic diuretics, carbonic anhydrase inhibitors, loop diuretics, thiazides, and K+-sparing agents

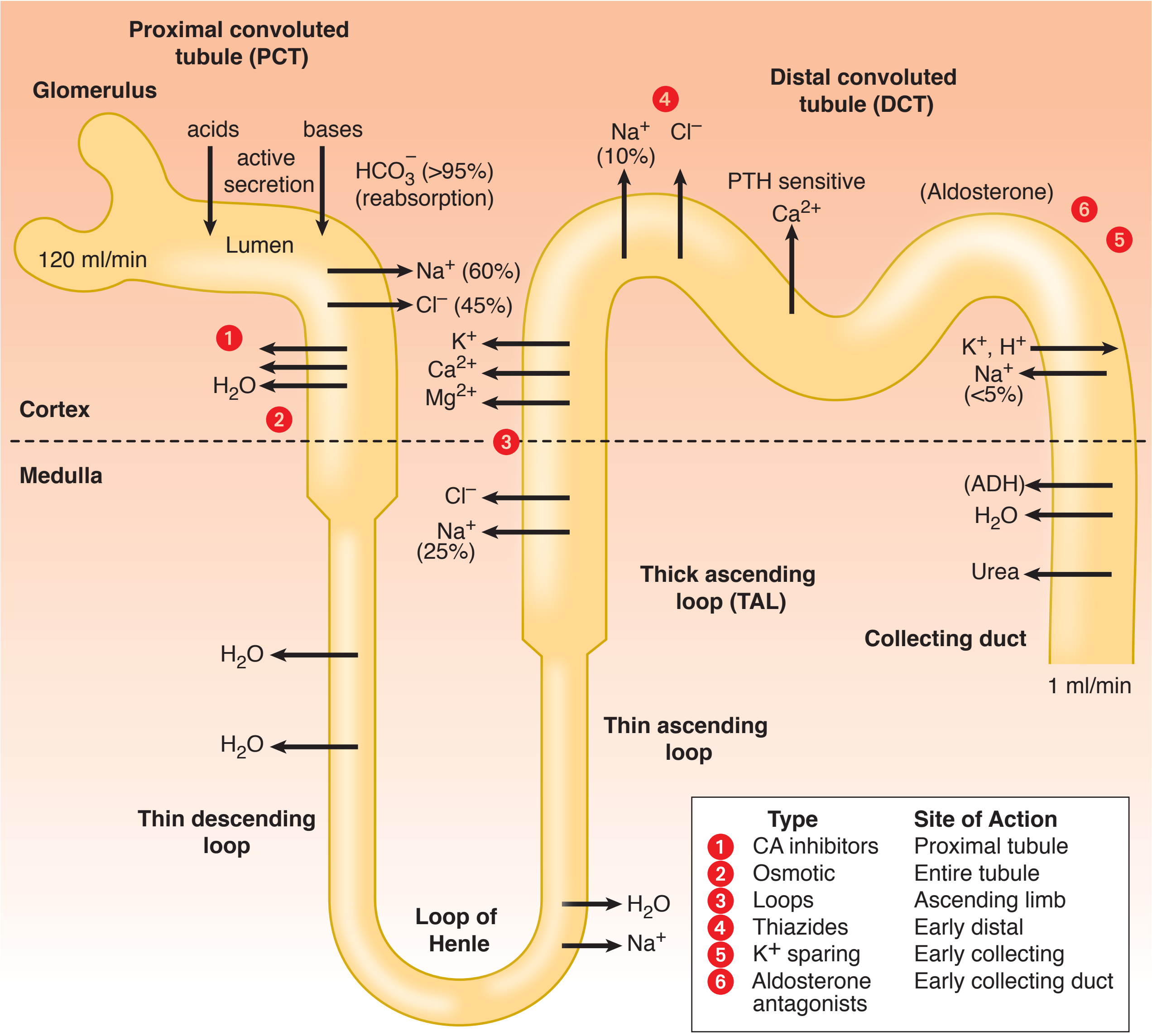


Figure III-1-1. Actions of Diuretics at the Various Renal Tubular Segments

Clinical Correlate

Osmotic diuretics are contraindicated in CHF and pulmonary edema because they draw water from the cells and increase the filling pressure of the heart.

TYPES OF DIURETICS

Osmotic Diuretics

- Mannitol (IV) inhibits water reabsorption throughout the tubule.
- It increases urine volume.
- Uses:
  - ↓ IOP in glaucoma
  - ↓ intracerebral pressure
  - Oliguric states (e.g., rhabdomyolysis)
- Side effects: acute hypovolemia

Carbonic Anhydrase Inhibitors

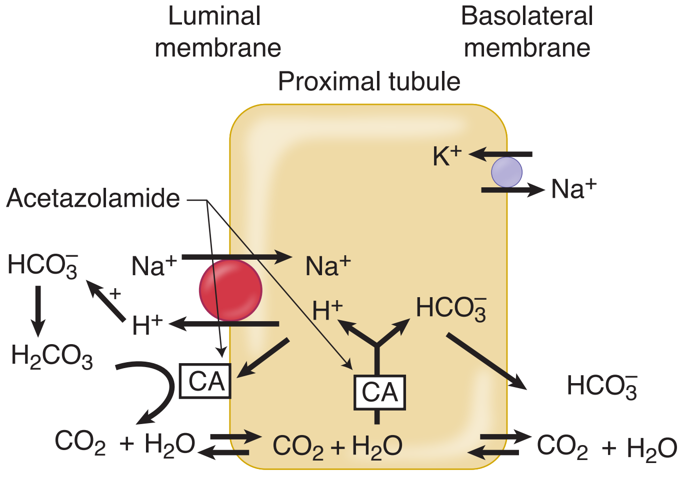


Figure III-1-2. Actions of Carbonic Anhydrase Inhibitors

- Drugs: **acetazolamide** and **dorzolamide**
- Mechanism: carbonic anhydrase inhibition, results in:
  - ↓ H^+ formation inside PCT cell
  - ↓ Na^+/H^+ antiport
  - ↑ Na^+ and HCO_3^- in lumen
  - ↑ diuresis
- Uses:
  - Glaucoma
  - Acute mountain sickness
  - Metabolic alkalosis
- Side effects:
  - Bicarbonaturia and acidosis
  - Hypokalemia

- Hyperchloremia
- Paresthesias
- Renal stones
- Sulfonamide hypersensitivity

Loop Diuretics

High-Yield

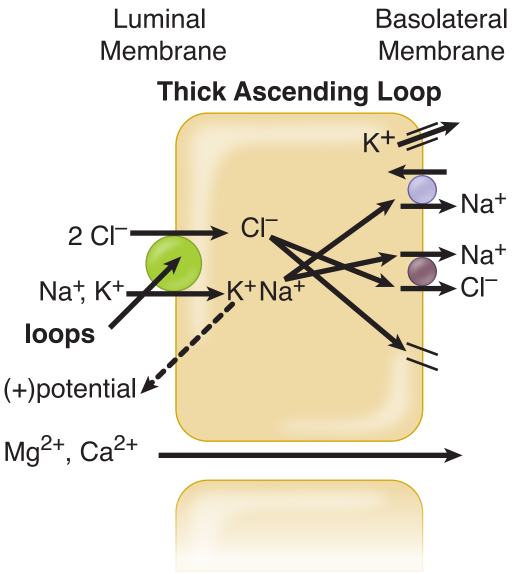


Figure III-1-3. Actions of Loop Diuretics on the Thick Ascending Loop (TAL)

- Drugs: **furosemide**, **torsemide**, and **ethacrynic acid**
- Mechanism: $Na^+/K^+/2Cl^-$ transporter inhibition, results in:
  - ↓ intracellular K^+ in TAL
  - ↓ back diffusion of K^+
  - ↓ positive potential
  - ↓ reabsorption of Ca^{2+} and Mg^{2+}
  - ↑ diuresis
- Uses:
  - Acute pulmonary edema
  - Heart failure
  - Hypertension
  - Refractory edemas
  - Anion overdose
  - Hypercalcemic states

Note

Sulfonamide-containing drugs have cross allergenicity with:

- Carbonic anhydrase inhibitors
- All loop diuretics (except ethacrynic acid)
- Thiazides
- Sulfa antibiotics
- Celecoxib

- Side effects:
  - Sulfonamide hypersensitivity (furosemide)
  - Hypokalemia and alkalosis
  - Hypocalcemia
  - Hypomagnesemia
  - Hyperuricemia (actively secreted by the OAT)
  - Ototoxicity (ethacrynic acid > furosemide)
- Drug interactions
  - Aminoglycosides (enhanced ototoxicity)
  - Lithium (chronic loop administration, ↓ clearance)
  - Digoxin (↑ toxicity due to electrolyte disturbances)

Clinical Correlate

An important difference between loops and thiazides is that loops **promote** calcium $^{+}$excretion, while thiazides **decrease** calcium excretion.

Clinical Correlate

Thiazides also hyperpolarize both smooth muscle cells (vasodilation) and pancreatic beta cells (decrease insulin release)

Thiazides

High-Yield

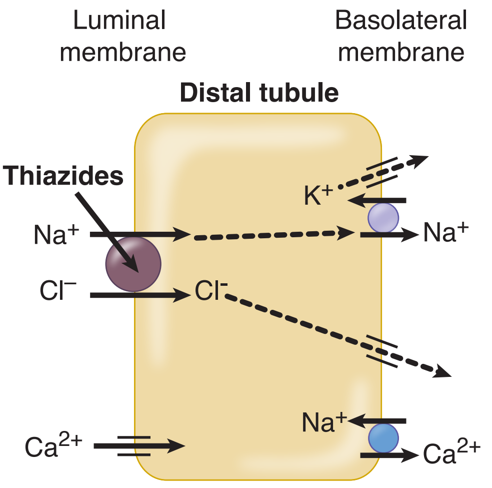


Figure III-1-4. Actions of Thiazides on the Distal Convoluted Tubule (DCT)

- Drugs: **hydrochlorothiazide**, **chlorthalidone**, and **indapamide**
- Mechanism: Na^+/Cl^- transporter inhibition, results in:
  - ↑ luminal Na^+ and Cl^- in DCT
  - ↑ diuresis
- Uses:
  - Hypertension, CHF
  - Nephrolithiasis (calcium stones)
  - Nephrogenic diabetes insipidus
- Side effects:
  - Sulfonamide hypersensitivity
  - Hypokalemia and alkalosis

  - Hypercalcemia
  - Hyperuricemia (actively secreted by the OAT)
  - Hyperglycemia
  - Hyperlipidemia (except indapamide)
- Drug interactions and cautions:
  - Digoxin (↑ toxicity due to electrolyte disturbances)
  - Avoid in patients with diabetes mellitus

Recall Question

Furosemide inhibits water reabsorption at which part of the renal anatomy?

A. Collecting duct

B. Thin ascending loop

C. Thin descending loop

D. Thick ascending loop

Answer: D

K^+-Sparing Agents

High-Yield

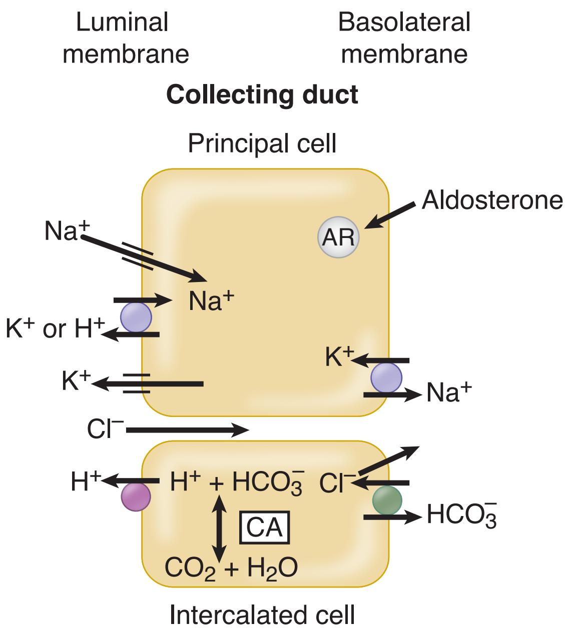


Figure III-1-5. Actions of Potassium-Sparing Agents on Collecting Tubules

Clinical Correlate

Combining K^+-sparing diuretics with ACEIs or ARBs may cause hyperkalemia.

Note

Diuretics that block Na^+ reabsorption at segments above the collecting ducts will increase sodium load to the collecting tubules and ducts ("downstream"). This results in increased loss of K^+ → **hypokalemia**. In the case of both loop and thiazide diuretics, the associated loss of H^+ results in **alkalosis**.

Note

Eplerenone is a selective aldosterone receptor blocker devoid of antiandrogenic effect.

- Drugs:
  - **Spironolactone:** aldosterone-receptor antagonist
    - Uses:
      - Hyperaldosteronic state
      - Adjunct to K^+-wasting diuretics
      - Antiandrogenic uses (female hirsutism)
      - Congestive heart failure
    - Side effects: hyperkalemia and acidosis; antiandrogen
  - Amiloride and triamterene: Na^+-channel blockers
    - Uses: adjunct to K^+-wasting diuretics, lithium-induced nephrogenic diabetes insipidus (amiloride)
    - Side effects: hyperkalemia and acidosis

Table III-1-1. Modes of Action and Effects of Various Classes of Diuretics

Drug	Mechanisms of Action	Urinary Electrolytes	Blood pH
Acetazolamide	Inhibition of carbonic anhydrase in PCT	↑ Na^+ ↑ K^+ ↑↑ HCO_3^-	Acidosis
Ethacrynic acid, furosemide, torsemide	Inhibition of $Na^+/K^+/2Cl^-$ cotransporter in TAL	↑↑ Na^+ ↑ K^+ ↑ Ca^{2+} ↑ Mg^{2+} ↑ Cl^-	Alkalosis
Hydrochlorothiazide, indapamide, chlorthalidone	Inhibition of Na^+/Cl^- cotransporter in DCT	↑ Na^+ ↑ K^+ ↑ Cl^- ↓ Ca^{2+}	Alkalosis
Amiloride, triamterene, spironolactone, eplerenone	Block Na^+ channels, block aldosterone receptors in collecting tubule	↑ Na^+ (small) ↓ K^+	Acidosis

2 Antihypertensives

Learning Objectives

- ❑ Differentiate between angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers
- ❑ Explain drug strategy for treating hypertension using calcium-channel blockers, drugs altering sympathetic activity, and direct-acting vasodilators
- ❑ Answer questions about indications for use of antihypertensive drugs
- ❑ Describe modifications of hypertension treatment in comorbid conditions
- ❑ Apply knowledge of treatment of pulmonary hypertension

DRUG STRATEGY

- ↓ TPR
- ↓ CO
- ↓ body fluid volume
- ↓ BP may result in homeostatic regulation:
  - Reflex tachycardia (↑ sympathetic activity)
  - Edema (↑ renin activity)

Clinical Correlate

Current recommendations are to use thiazide diuretics, ACEIs, or long-acting CCBs as first-line therapy. These drugs are considered equally effective.

FIRST-LINE ANTIHYPERTENSIVES

Thiazide Diuretics

High-Yield

Thiazide diuretics are commonly used in the management of hypertension.

Angiotensin-Converting Enzyme Inhibitors (ACEIs) and Angiotensin-Receptor Blockers (ARBs)

High-Yield

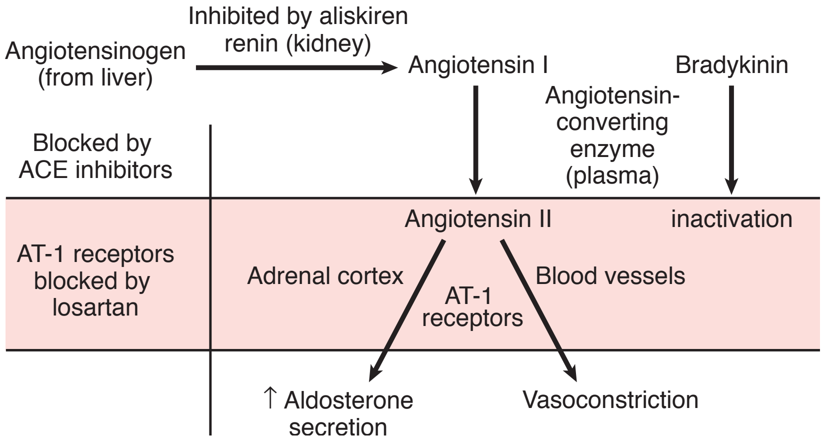


Figure III-2-1. The Angiotensin System

- Drugs:
  - ACEIs: **captopril, lisinopril** (and other "–prils")
    - Block formation of angiotensin II
    - Resulting in prevention of AT_1-receptor stimulation
    - ↓ aldosterone, vasodilation
    - ACEIs prevent bradykinin degradation
  - ARBs: **losartan** (and other "–sartans")
    - Block AT_1 receptors
    - Same results as ACEIs on BP mechanisms
    - ARBs do not interfere with bradykinin degradation
  - Renin inhibitor: Aliskiren
    - Blocks formation of angiotensin I
    - Same results as ACEIs on BP mechanisms
    - Aliskiren does not interfere with bradykinin degradation
- Uses:
  - Mild-to-moderate hypertension (all)
  - Protective of diabetic nephropathy (ACEI/ARBs)
  - CHF (ACEI/ARBs)

- Side effects:
  - Dry cough (ACEIs)
  - Hyperkalemia
  - Acute renal failure in renal artery stenosis
  - Angioedema
- Contraindication: pregnancy

Calcium-Channel Blockers

High-Yield

Calcium-channel blockers (CCBs) block L-type Ca^{2+} channels in heart and blood vessels.

- Results in ↓ intracellular Ca^{2+}
- Causes ↓ CO (verapamil and diltiazem), ↓ TPR (all CCBs)
- Drugs: verapamil, diltiazem, **dihydropyridines** (–“dipines,” prototype: **nifedipine**)

Figure III-2-2. Cardiac or Vascular Selectivity of Major Ca^{2+}-Channel Blockers

- Uses:
  - Hypertension (all drugs)
  - Angina (all drugs)
  - Antiarrhythmics (verapamil, diltiazem)
- Side effects:
  - Reflex tachycardia (“–dipines”)
  - Gingival hyperplasia (“–dipines”)
  - Constipation (verapamil)

Bridge to Physiology

Vasodilators may have specificity.

- Arteriolar: Ca^{2+}-channel blockers, hydralazine, K^{+}-channel openers
- Venular: nitrates
- Both arteriolar and venular: “the rest”

Orthostatic (postural) hypotension results from venular dilation (not arteriolar) and mainly results from α_1 blockade or decreased sympathetic tone.

DRUGS ALTERING SYMPATHETIC ACTIVITY

Beta Blockers

- Mechanism (*See* ANS section)
  - Side effects:
    - Cardiovascular depression
    - Fatigue
    - Sexual dysfunction
    - ↑ LDLs and TGs
  - Cautions in use:
    - Asthma
    - Vasospastic disorders
    - Diabetics (alteration of glycemia and masking of tachycardia due to hypoglycemic events)

Alpha-1 Blockers

- Decrease arteriolar and venous resistance
- Reflex tachycardia
- Drugs: **prazosin, doxazosin, terazosin**
- Uses:
  - Hypertension
  - BPH: ↓ urinary frequency and nocturia by ↓ the tone of urinary sphincters
- Side effects: "first-dose" syncope; orthostatic hypotension; urinary incontinence
- Advantage: good effect on lipid profile (↑ HDL, ↓ LDL)

Alpha-2 Agonists

- Drugs: **clonidine** and **methyldopa** (prodrug)
- α_2 stimulation: ↓ in sympathetic outflow; ↓ TPR but also ↓ HR
- Uses:
  - Mild-to-moderate hypertension (both)
  - Opiate withdrawal (clonidine)
  - Hypertensive management in pregnancy (methyldopa)
- Side effects: positive Coombs test (methyldopa); CNS depression (both); edema (both)
- Drug interactions: tricyclic antidepressants ↓ antihypertensive effects of α_2 agonists

DIRECT-ACTING VASODILATORS

Drugs Acting Through Nitric Oxide

- **Hydralazine**
  - ↓ TPR via arteriolar dilation
  - Use: moderate-to-severe hypertension
  - Side effects: SLE-like syndrome and slow acetylators; edema; reflex tachycardia
- **Nitroprusside**
  - ↓ TPR via dilation of both arterioles and venules
  - Use: hypertensive emergencies (used IV)
  - Side effects: cyanide toxicity (co-administered with nitrites and thiosulfate)

Drugs Acting to Open Potassium Channels

- Drugs: **minoxidil** and diazoxide
  - Open K^+ channel, causing hyperpolarization of smooth muscle
  - Results in arteriolar vasodilation
  - Uses:
    - Insulinoma (diazoxide)
    - Severe hypertension (minoxidil)
    - Baldness (topical minoxidil)
  - Side effects:
    - Hypertrichosis (minoxidil)
    - Hyperglycemia (↓ insulin release [diazoxide])
    - Edema
    - Reflex tachycardia

Clinical Correlate

Chronic (preexisting) hypertension in pregnancy is often treated with methyldopa or labetalol, while preeclampsia (new-onset hypertension in pregnancy) is treated with labetalol or hydralazine.

Clinical Correlate

Sodium nitrite or amyl nitrite can be used in **cyanide poisoning**.

- Promotes formation of methemoglobin (MetHb), which binds CN^- ions, forming cyanomethemoglobin
- Prevents the inhibitory action of CN^- on complex IV of electron transport chain
- Cyanomethemoglobin is then reconverted to methemoglobin by treatment with sodium thiosulfate, forming the less toxic thiocyanate ion (SCN^-)
- MetHb is converted to oxyhemoglobin with methylene blue

Clinical Correlate

A hypertensive emergency occurs when hypertension is severe enough to cause end-organ damage. Most commonly, nitroprusside, labetalol, or the D1 agonist fenoldopam is given intravenously as therapy.

INDICATIONS FOR USE OF ANTIHYPERTENSIVE DRUGS IN COMORBID CONDITIONS

Table III-2-1. Use of Antihypertensive Drugs in Comorbid Conditions

Indication	Suitable Drug(s)
Angina	Beta blockers, CCBs
Diabetes	ACEIs, ARBs
Heart failure	ACEIs, ARBs, beta blockers
Post-MI	Beta blockers
BPH	Alpha blockers
Dyslipidemias	Alpha blockers, CCBs, ACEIs/ARBs
Chronic kidney disease	ACEI, ARBs

Recall Question

Angiotensin converting enzyme inhibitors (ACEIs) have the side effect of dry cough due to preventing degradation of what substance?

A. Angiotensinogen

B. Angiotensin I

C. Bradykinin

D. Renin

Answer: C

TREATMENT OF PULMONARY HYPERTENSION

Bosentan

Endothelin (ET)-1 is a powerful vasoconstrictor through ET-A and -B receptors. Bosentan is an ET-A receptor antagonist.

- Administered orally
- Side effects: those associated with vasodilation (headache, flushing, hypotension)
- Contraindication: pregnancy

Prostacyclin (PGI_2): Epoprostenol

- Administered via infusion pumps

Sildenafil

- Inhibits type V PDE
- Increases cGMP
- Pulmonary artery relaxation
- Decreases pulmonary hypertension

Drugs for Heart Failure 3

Learning Objectives

- ❑ Describe the primary treatments for CHF
- ❑ Demonstrate understanding of inotropes
- ❑ Demonstrate understanding of other drugs used in CHF

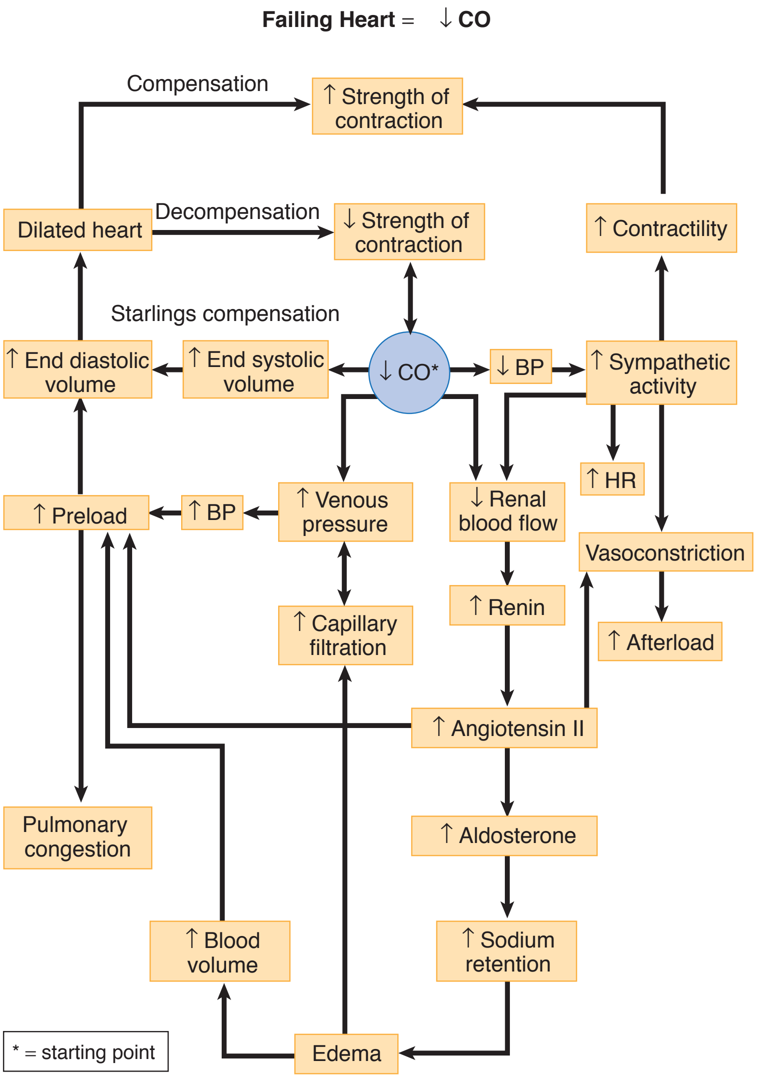


Figure III-3-1. The Failing Heart

Clinical Correlate

Left systolic dysfunction secondary to coronary artery disease is the most common cause of heart failure.

Pharmacotherapy aimed at:

- ↓ preload: diuretics, ACEIs, ARBs, and venodilators
- ↓ afterload: ACEIs, ARBs, and arteriodilators
- ↑ contractility: digoxin, beta agonists, PDE III inhibitors
- ↓ remodeling of cardiac muscle: ACEIs, ARBs, spironolactone, beta blockers

Whereas digoxin does not improve survival, ACEIs, ARBs, beta blockers, and spironolactone have been proven beneficial in CHF. ACEIs and ARBs are currently drugs of choice for the chronic management of CHF. Inotropes are more beneficial in management of acute CHF.

PRIMARY TREATMENTS FOR CHF

- ACEI (ARB as an alternative)
- Beta blockers (metoprolol, bisoprolol, carvedilol)
  - Provide antiarrhythmic effect and also ↓ remodeling
- Diuretics
  - Loop or thiazide diuretics to decrease preload
  - Spironolactone or eplerenone to block aldosterone receptors and ↓ remodeling (used in advanced CHF)
- Hydralazine + isosorbide dinitrate
  - Preferred for chronic therapy in patients who cannot tolerate an ACEI or ARB

INOTROPES

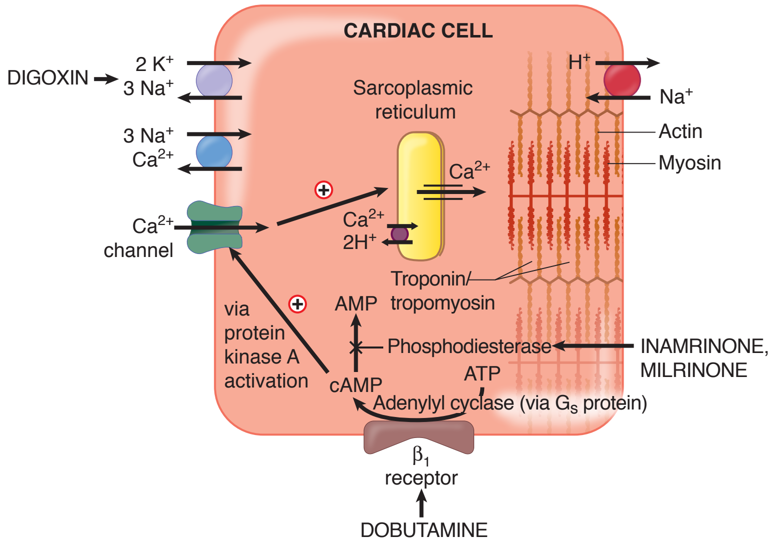


Figure III-3-2. Mechanism of Action of Inotropes

Digoxin

High-Yield

- Direct effect: inhibition of cardiac Na^+-K^+ ATPase
  - Results in ↑ intracellular Na^+
  - Decreases Na^+/Ca^{2+} exchange
  - Increases intracellular Ca^{2+}
  - Increases Ca^{2+} release from sarcoplasmic reticulum
  - Increases actin-myosin interaction
  - Increases contractile force
- Indirect effect: inhibition of neuronal Na^+-K^+ ATPase (results in ↑ vagal activity)
- Pharmacokinetics:
  - Renal clearance: caution in renal impairment
  - Long $t_{1/2}$: need loading dose (LD)
  - Tissue protein binding (large V_d): displacement by other drugs (verapamil, quinidine)
- Uses: CHF; supraventricular tachycardias, except Wolff-Parkinson-White syndrome
- Side effects:
  - Early signs include anorexia, nausea, ECG changes
  - Later signs include disorientation, visual effects (halos)
  - In toxic doses, any cardiac arrhythmias
- Management of toxicity: use of Fab antibodies toward digoxin; supportive therapy (electrolytes and antiarrhythmics class IB)
- Drug interactions:
  - Diuretics: ↓ K^+, ↓ Mg^{2+}, ↑ Ca^{2+}
  - Quinidine and verapamil

Phosphodiesterase Inhibitors: Inamrinone and Milrinone

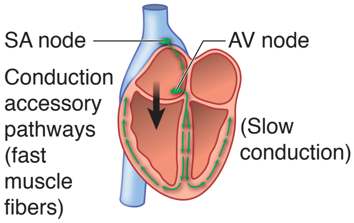
- Use: acute CHF only
- Increase cAMP in heart muscle; results in ↑ inotropy
- Increase cAMP in smooth muscle; results in ↓ TPR

Sympathomimetics: Dobutamine and Dopamine

- Use: acute CHF only

Note

Wolff-Parkinson-White Syndrome



- **Do** block accessory pathway with I_A or III
- **Do not** slow AV conduction (avoid digoxin, β-blocker, Ca^{2+}-channel blocker, adenosine)

Clinical Correlate

Diastolic dysfunction (CHF with preserved ejection fraction) is best treated with β blockers and diuretics.

Antiarrhythmic Drugs 4

Learning Objectives

- ❑ Demonstrate understanding of cardiac action potential
- ❑ Use knowledge of Na^+ channels to explain arrhythmias,
- ❑ Explain information related to ANS regulation of heart rate
- ❑ Answer questions about controlling arrhythmias using Na^+ channel blockers, beta blockers, K^+ channel blockers, Ca^{2+} channel blockers, and other unclassified drugs

CARDIAC ACTION POTENTIAL

Fast-Response Fibers: Cardiac Muscle, His-Purkinje System

High-Yield

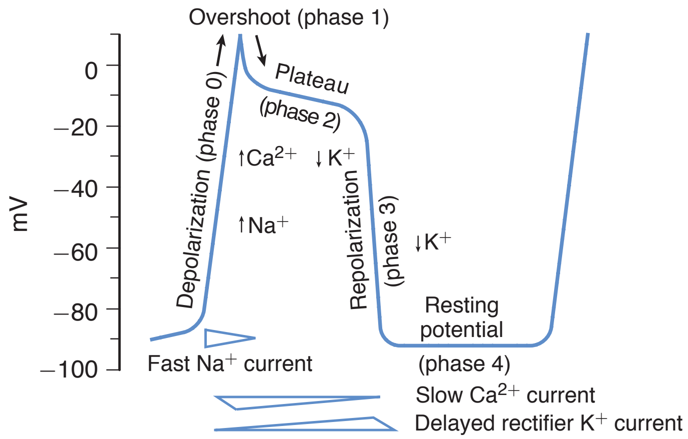


Figure III-4-1. Cardiac Action Potentials in Fast-Response Fibers

Phase 0

- Na^+ channels open: sodium enters the cell down its concentration gradient (fast I_{Na}), causing membrane depolarization.
- Rate of depolarization depends on number of Na^+ channels open, which in turn depends on resting membrane potential of the cell.
- Class I antiarrhythmic drugs can slow or block phase 0 in fast-response fibers.

Phase 1

- Na^+ channels are inactivated.
- In some His-Purkinje cells, transient outward K^+ currents and inward Cl^- currents contribute to the "notch" and overshoot.
- Antiarrhythmic drugs have no significant effects on these transient currents.

Phase 2

- Plateau phase in which a slow influx of Ca^{2+} ($I_{Ca\text{-}L}$) is "balanced" by a late-appearing outward K^+ current (the delayed rectifier current I_K).
- Antiarrhythmic drugs have no significant effects on these currents during this phase of the action potential (AP).

Phase 3

- Repolarization phase in which the delayed rectifier K^+ current rapidly increases as the Ca^{2+} current dies out because of time-dependent channel inactivation.
- Class III antiarrhythmic drugs slow this repolarization phase.
- Note that during phases 0 through 3 a slow Na^+ current ("window current") occurs, which can help prolong the duration of the action potential.

Phase 4

- Return of membrane to resting potential—maintained by activity of the Na^+/K^+-ATPase.

Responsiveness

- Capacity of a cell to depolarize, associated with the number of Na^+ channels in a ready state (*see* Figure III-4-4).
- This in turn depends on resting membrane potential: the more negative the resting potential (RP), the faster the response.

Conductance

Rate of spread of an impulse, or conduction velocity—three major determinants:

- Rate of phase 0 depolarization—as V_{max} decreases, conduction velocity decreases and vice versa.
- Threshold potential—the less negative, the slower the conduction velocity.
- Resting potential—the more negative the RP, the faster the conduction.

Slow-Response Fibers (SA and AV Nodes, Specialized Cells)

High-Yield

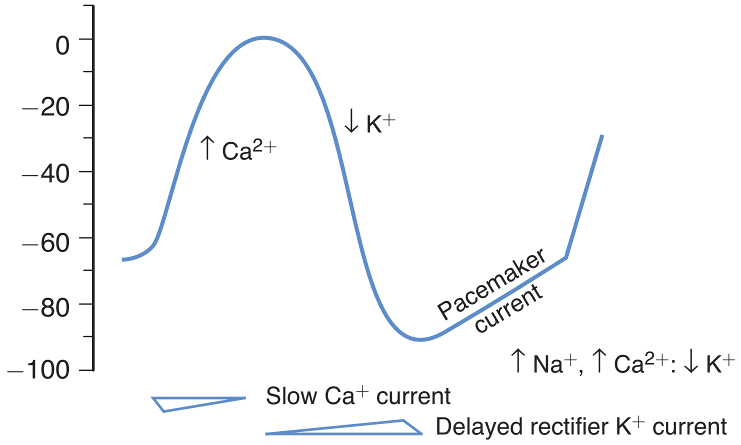


Figure III-4-2. Cardiac Action Potentials in Slow-Response Fibers

- No appreciable Na^+ current during phase 0 in these cells because the Na^+ channels are either absent or in an inactive form because of the existing voltage.
- Depolarization depends on activation of Ca^{2+} channels ($I_{Ca\text{-}L}$ and $I_{Ca\text{-}T}$).
- Class IV antiarrhythmic drugs can slow or block phase 0 in slow-response fibers.
- During repolarization, the Ca^{2+} currents are opposed and overcome by the delayed rectifier K^+ current. The relative magnitudes of these opposing currents determine the "shape" of the action potential.
- The major distinctive feature of slow fibers is their spontaneous depolarization, shown by the rising slope of phase 4 of the AP, referred to as the pacemaker potential or "pacemaker current." Although not completely understood, pacemaker potential is a composite of inward Na^+ (I_f) and Ca^{2+} ($I_{Ca\text{-}T}$) currents and outward K^+ currents (I_K).
- Class II and IV antiarrhythmic drugs can slow phase 4 in pacemaker fibers.

Automaticity

The ability to depolarize spontaneously confers automaticity on a tissue. The fastest phase 4 slope will determine the pacemaker of the heart (normally the SA node).

Refractoriness

Refractoriness is the inability to respond to a stimulus—a property of all cardiac cells.

Effective Refractory Period (ERP)

- No stimulus, of any magnitude, can elicit a response.
- Lasts into late stage 3 of the AP because Na^+ channels are effectively inactivated and not in the "ready" state.
- Blockers of K^+ channels prolong the ERP.

Relative Refractory Period (RRP)

- A strong stimulus can elicit a response, but the timing will be out of sync with the rest of the heart and arrhythmias may occur.
- Ratio of ERP to the action potential duration (APD) is a measure of refractoriness, as illustrated in Figure III-4-3. Decreases in ERP favor the formation and propagation of premature impulses.

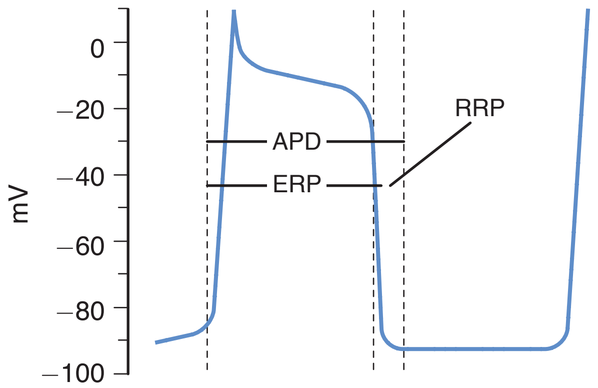


Figure III-4-3. Relationship of ERP to APD

NA$^+$ CHANNELS

Activation

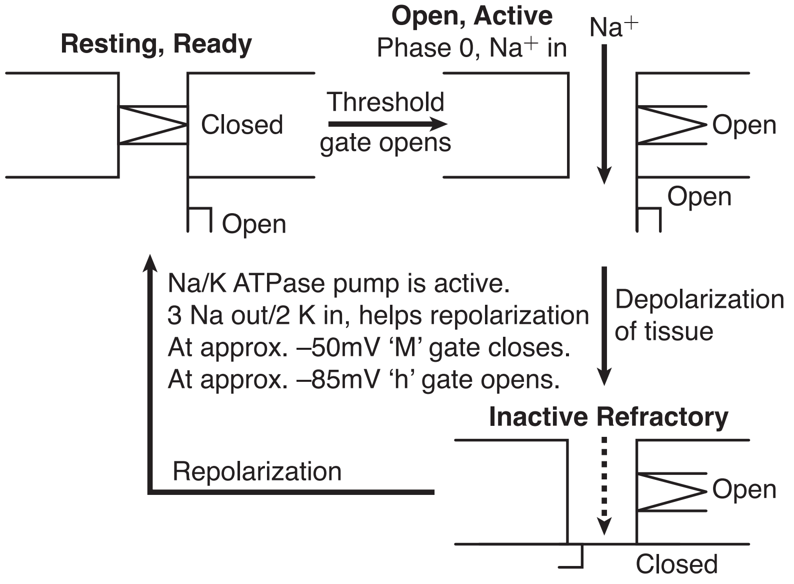


Figure III-4-4. Mechanism of Action of Voltage-Gated Na$^+$ Channels

- This voltage-gated channel, which is responsible for the fast Na$^+$ current (I_{Na}), exists in 3 conformations: resting or ready state; open or active state; and inactivated or refractory state.
- The channel has 2 gates: M (activating) and h (inactivating), both of which are sensitive to voltage changes.
- Inactivation of the h gate is slower; therefore, it stays open longer and the Na$^+$ channel is active.

Recovery

Rate of recovery of the Na$^+$ channel is dependent on the resting potential (RP).

- Fastest rate of recovery occurs at normal RP, and recovery slows as membrane voltage increases.
- Rate of recovery is slower in ischemic tissue because cells may be partly depolarized at rest. This reduces the number of channels able to participate in the next depolarization, which leads to a decrease in conduction rate in ischemic tissue.
- Na$^+$ channel blockers also slow the rate of recovery in such tissues.

ANS REGULATION OF HEART RATE

Nodal tissue, especially that of the SA node, is heavily innervated by both PANS and SANS fibers activating M_2 and β_1 receptors, respectively. Phase 4 slope is increased by an increase in cAMP resulting from β_1 receptor activation and slowed by a decrease in cAMP resulting from M_2 receptor activation.

- Increase in cAMP will:
  - Increase upstroke velocity in pacemakers by increase of $I_{Ca\text{-}L}$
  - Shorten AP duration by increase of I_K
  - Increase HR by increase of I_f, thus increasing slope of phase 4
- Decrease in cAMP:
  - Does the opposite plus produces a K^+ current ($I_{K/ACh}$), which slows the rate of diastolic depolarization and thus decreases HR
  - Beta blockers prevent cAMP formation, with primary effects on SA and AV nodal tissues.

CLASS I: Na^+ CHANNEL BLOCKERS

Class 1A

High-Yield

- Antiarrhythmic: block fast Na^+ channels (↓ I_{Na})
- Preferentially in the open or activated state—“state-dependent” blockade
- Also blocks K^+channel (prolongs repolarization), ↑ action potential duration and effective refractory period
- Drugs:
  - **Quinidine**
    - In addition to the above, causes muscarinic receptor blockade, which can ↑ HR and AV conduction.
    - May also cause vasodilation via alpha block with possible reflex tachycardia.
    - Orally effective, wide clinical use in many arrhythmias; in atrial fibrillation, need initial digitalization to slow AV conduction.
    - Adverse effects: cinchonism (GI, tinnitus, ocular dysfunction, CNS excitation), hypotension, prolongation of QRS and ↑ QT interval associated with syncope (torsade).
    - Drug interactions: hyperkalemia enhances effects and vice versa; displaces digoxin from tissue binding sites, enhancing toxicity.
  - **Procainamide**
    - Less muscarinic receptor block
    - Metabolized via *N*-acetyltransferase (genotypic variation) to *N*-acetyl procainamide (NAPA), an active metabolite
    - Adverse effects: systemic lupus erythematosus (SLE)–like syndrome (30% incidence) more likely with slow acetylators; hematotoxicity (thrombocytopenia, agranulocytosis); CV effects (torsade)

Note

For the exam, know which effect is **antiarrhythmic** (eliminates irregular heartbeat) and which is **proarrhythmic** (promotes irregular heartbeat).

Note

Quinidine is a weak base, and antacids increase its absorption, thus greatly increasing its toxicity.

Class 1B

- Antiarrhythmic: block fast Na^+ channels (↓ I_{Na})
- Block inactivated channels—preference for tissues partly depolarized (slow conduction in hypoxic and ischemic tissues). This results in an increased threshold for excitation and less excitability of hypoxic heart muscle.
- ↓ APD—due to block of the slow Na^+ "window" currents, but this increases diastole and extends the time for recovery.
- Drugs and uses:
  - **Lidocaine**
    - Post-MI, open-heart surgery, digoxin toxicity–ventricular arrhythmias only
    - Side effects: CNS toxicity (seizures); least cardiotoxic of conventional anti-arrhythmics
    - IV use because of first-pass metabolism
  - **Mexiletine**
    - Same uses as lidocaine
    - Oral formulations

Class 1C

- Block fast Na^+ channels (↓ I_{Na}), especially His-Purkinje tissue
- No effect on APD
- No ANS effects
- Drug:
  - **Flecainide**
    - Limited use because of proarrhythmogenic effects, leading to ↑ in sudden death post-MI and when used prophylactically in VT

CLASS II: BETA BLOCKERS

- Prevent β-receptor activation, which would normally ↑ cAMP
- ↓ SA and AV nodal activity
- ↓ Slope of phase 4 (diastolic currents) of AP in pacemakers
- Drugs:
  - Propranolol (nonselective) and the cardioselective drugs: acebutolol and esmolol
  - Uses:
    - Prophylaxis post-MI and in supraventricular tachyarrhythmias (SVTs)
    - Esmolol (IV) is used in acute SVTs

CLASS III: K^+ CHANNEL BLOCKERS

- Decrease I_K (delayed rectifier current) slowing phase 3 (repolarization) of AP
- Increase APD and ERP, especially in Purkinje and ventricular fibers

Amiodarone

High-Yield

- Mimics classes I, II, III, and IV
- Increase APD and ERP in all cardiac tissues
- Uses: any arrhythmia
- $t_{1/2}$ >80 days
- Binds extensively to tissues (large V_d and multiple effects)
- Side effects: pulmonary fibrosis, blue pigmentation of the skin ("smurf skin"), phototoxicity, corneal deposits, hepatic necrosis, thyroid dysfunction

Sotalol

- Decreases IK, slowing phase III
- Non-selective beta blocker: β1 blockade, leading to ↓ HR, ↓ AV conduction
- Use: life-threatening ventricular arrhythmia
- Side effects: Torsade

CLASS IV: Ca^{2+} CHANNEL BLOCKERS

- Block slow cardiac Ca^{2+} channels
- Decrease phase 0, ↓ phase 4
- Decrease SA, ↓ AV nodal activity

Verapamil and Diltiazem

- Prototype Ca^{2+}-channel blockers (*see* Antihypertensive Drugs and Antianginal Drugs chapters in this section)
- Uses: supraventricular tachycardias
- Side effects: constipation (verapamil), dizziness, flushing, hypotension, AV block
- Drug interaction:
  - Additive AV block with β-blockers, digoxin
  - Verapamil displaces digoxin from tissue-binding sites

Clinical Correlate

Long QT Syndrome

A familial condition associated with increased risk of ventricular arrhythmias may result from a mutation in the gene encoding cardiac potassium channels.

In such patients, class IA and class III antiarrhythmic drugs may increase the risk of torsades. Drugs which cause torsades include:

- Potassium-channel blockers (class 1A and class III)
- Antipsychotics (thioridazine)
- Tricyclic antidepressants

To **treat the torsades**, correct the hypokalemia, correct the hypomagnesemia, and discontinue drugs that prolong the QT interval.

Clinical Correlate

Atrial fibrillation is the most common arrhythmia in the United States. There are 2 primary goals:

- Ventricular rate control with beta blocker, CCB, or digoxin
- Anticoagulation

UNCLASSIFIED

Adenosine

- Activates adenosine receptors: causes G_i-coupled decrease in cAMP
- Decreases SA and AV nodal activity
- Uses: DOC for paroxysmal supraventricular tachycardias and AV nodal arrhythmias
- Administered IV: $t_{1/2}$ <10 seconds
- Side effects: flushing, sedation, dyspnea
- Adenosine is antagonized by methylxanthines (theophylline and caffeine)

Magnesium

- Use: torsades

Recall Question

Which of the following drugs is associated with an SLE-like syndrome?

A. Captopril

B. Lidocaine

C. Procainamide

D. Quinidine

Answer: C

Clinical Correlate

Potassium

Both hyperkalemia and hypokalemia are arrhythmogenic.

Antianginal Drugs 5

Learning Objectives

- ❏ Solve problems concerning the rationale for the use of nitrates, beta blockers, and carvedilol for angina
- ❏ Use knowledge of calcium channel blockers
- ❏ Demonstrate understanding of ranolazine

RATIONALE FOR USE

Angina pectoris is the principal syndrome of ischemic heart disease, anginal pain occurring when oxygen delivery to the heart is inadequate for myocardial requirement.

- Stable/classic angina (angina of effort or exercise) is due to coronary atherosclerotic occlusion
- Vasospastic or variant angina (Prinzmetal) is due to a reversible decrease in coronary blood flow

Drug Strategies in Stable and Vasospastic Angina

Drug strategies in stable and vasospastic angina involve:

- ↓ oxygen requirement by ↓ TPR, CO, or both (nitrates, CCBs, and beta blockers).
- ↑ oxygen delivery by ↓ vasospasm (nitrates and CCBs).

KEY ANTIANGINAL DRUGS

Nitrates

High-Yield

Nitrates are prodrugs of nitric oxide.

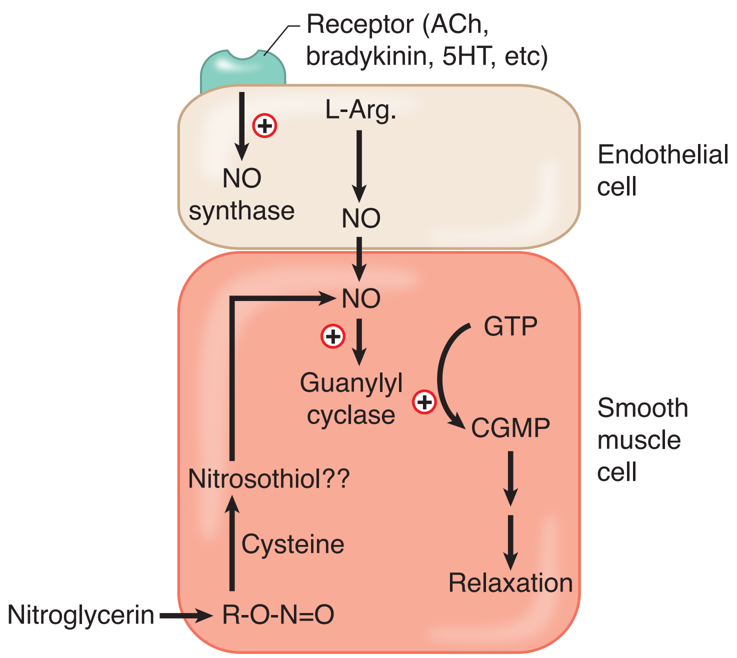


Figure III-5-1. Nitrates and the Nitric Oxide Pathway

Clinical Correlate

Sildenafil (Viagra)

Phosphodiesterase 5 (PDE5) is found in blood vessels supplying the corpora cavernosa. Sildenafil inhibits PDE 5 → ↑ cGMP → vasodilation → ↑ blood flow → ↑ erectile response.

If used concomitantly with nitrates or other potent vasodilators, the excessive fall in BP may lead to death from cardiovascular causes, including myocardial infarct.

- Venodilation →↓ preload →↓ cardiac work →↓ oxygen requirement
- Nitrates ↓ infarct size and post-MI mortality
- Drugs:
  - **Nitroglycerin:** sublingual, transdermal, and IV formulations
  - Isosorbide: oral, extended release for chronic use
  - Side effects:
    - Flushing, headache, orthostatic hypotension
    - Reflex tachycardia and fluid retention
  - Cautions and contraindications:
    - Tachyphylaxis with repeated use
    - Cardiovascular toxicity with sildenafil

Beta Blockers and Carvedilol

- Used in angina of effort
- β-blockers are contraindicated in vasospastic angina
- Carvedilol is clinically equivalent to isosorbide in angina of effort

Calcium Channel Blockers (CCBs)

- All CCBs can be used.
- Nifedipine is important for vasospastic angina.
- *See* Antihypertensive Drugs, chapter 3 in this section.

Ranolazine

- Ischemia causes increased sodium which prevents calcium exit through Na^+/Ca^{++} exchanger pump
- Ranolazine blocks late inward Na^+ current in cardiac myocytes, thereby decreasing calcium accumulation
- Results in decreased end diastolic pressure and improvement of diastolic coronary flow
- Side effects include constipation and nausea; increased QT makes the drug contraindicated in patients with long QT syndrome or taking drugs which increase QT (*see* Magnesium discussion in Chapter 4, Antiarrhythmic Drugs)

Clinical Correlate

Drugs that decrease mortality in patients with stable angina include aspirin, nitroglycerin, and beta blockers. Nitroglycerin is the preferred drug for acute management of both stable and vasospastic angina.

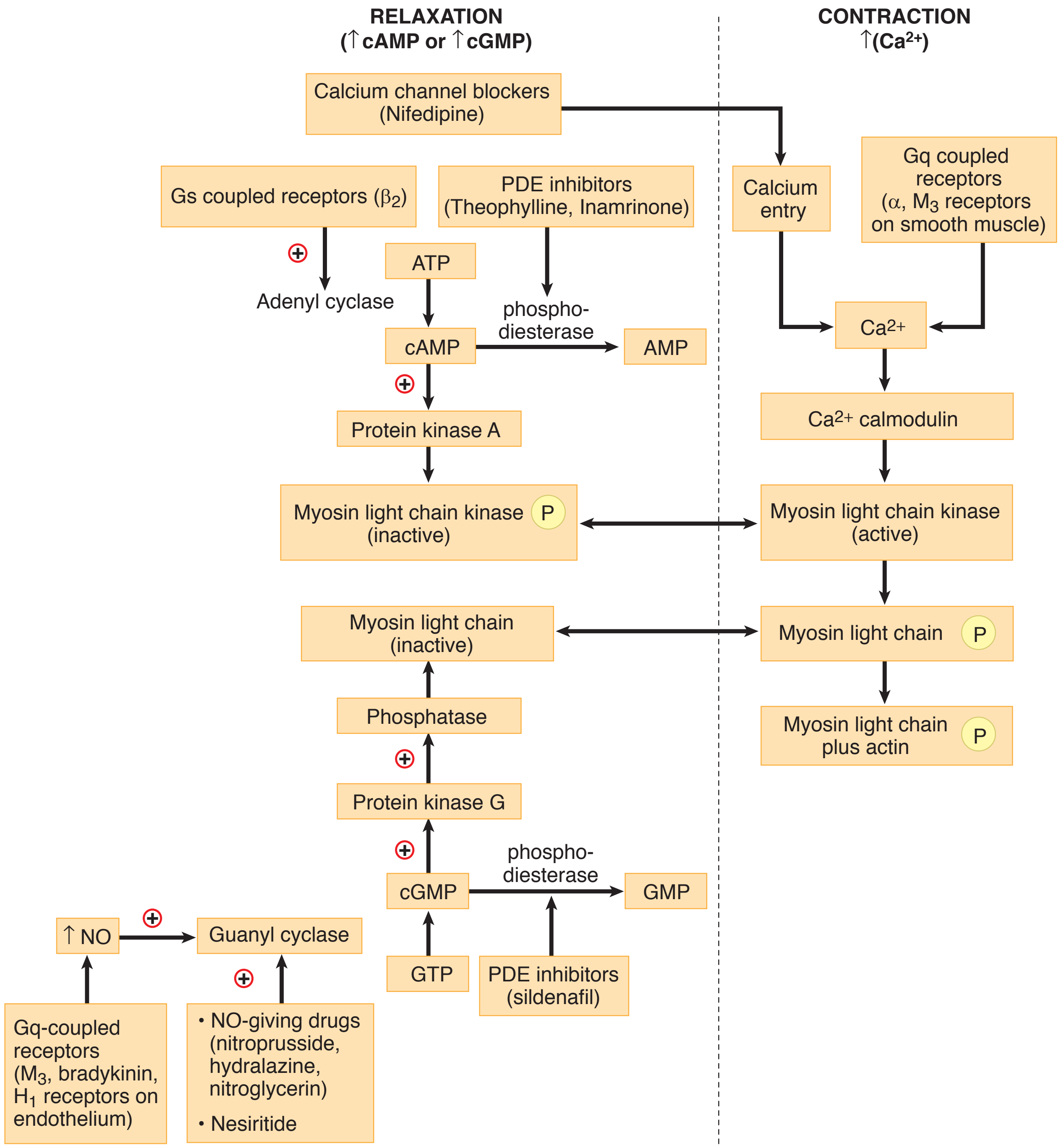


Figure III-5-2. Mechanisms of Smooth Muscle Contraction and Relaxation and Drugs Affecting Them

Antihyperlipidemics 6

Learning Objectives

- ❑ Solve problems concerning HMG-CoA reductase inhibitors
- ❑ Demonstrate understanding of bile acid sequestrants
- ❑ Use knowledge of nicotinic acid (niacin, vitamin B3)
- ❑ Solve problems concerning gemfibrozil, fenofibrate (fibrates)
- ❑ Explain information related to ezetimibe
- ❑ Answer questions related to orlistat

CARDIOVASCULAR RISKS OF HYPERLIPIDEMIA

- Increased risk of atherosclerosis is associated with hypercholesterolemia
- Increased risk of cardiovascular and cerebrovascular diseases
- Treatment goal is to ↓ LDL cholesterol and atheroma plaque formation

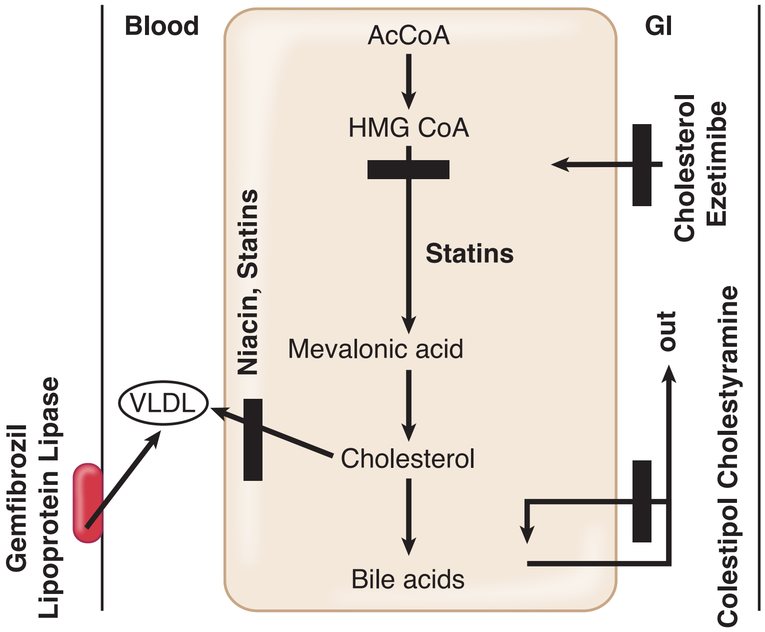


Figure III-6-1. Site of Action of Statins, Niacin, and Gemfibrozil on the Synthesis of Lipids

LIPID-LOWERING DRUGS

HMG-CoA Reductase Inhibitors

High-Yield

- Drugs: **atorvastatin, rosuvastatin,** and other "**–statins**"
  - At their highest therapeutic doses, atorvastatin and rosuvastatin are considered "high-intensity" statins and can lower LDL-C by ≥ 50%
  - Lower doses of statins are classified as "low" or "moderate" intensity
- Mechanisms:
  - HMG-CoA reductase inhibition, results in:
    - Decreased liver cholesterol
    - Increased LDL-receptor expression
    - Decreased plasma LDL
  - Decreased VLDL synthesis results in: ↓ triglyceridemia
- Side effects:
  - Myalgia, myopathy (check creatine kinase)
  - Rhabdomyolysis
  - Hepatotoxicity (check liver function tests)
- Drug interaction:
  - Gemfibrozil (↑ rhabdomyolysis)
  - Cytochrome P450 inhibitors enhance toxicity of statins

Bile Acid Sequestrants

- Drugs: **cholestyramine** and **colestipol**
- Mechanism: complexation of bile salts in the gut, results in:
  - Decrease enterohepatic recirculation of bile salts
  - Increase synthesis of new bile salts by the liver
  - Decrease liver cholesterol
  - Increase LDL-receptor expression
  - Decrease blood LDL
- Side effects:
  - Increase VLDL and triglycerides
  - Gastrointestinal disturbances
  - Malabsorption of lipid-soluble vitamins
  - Hyperglycemia
- Drug interactions with orally administered drugs (warfarin, thiazides, digoxin, etc.)
- Contraindication: hypertriglyceridemia

Clinical Correlate

Nonstatin drugs have not been shown to improve cardiovascular outcomes when added to statin therapy. These drugs are most often used in patients who cannot tolerate a statin.

Nicotinic Acid (Niacin, Vitamin B3)

- Mechanism: inhibition of VLDL synthesis, results in:
  - Decreased plasma VLDL
  - Decreased plasma LDL
  - Increased plasma HDL
- Side effects:
  - Flushing, pruritus, burning pain (use aspirin)
  - Hepatotoxicity
  - Hyperglycemia

Gemfibrozil, Fenofibrate (Fibrates)

- Mechanism: bind to the PPARα and increase expression of lipoprotein lipases, results in:
  - Decreased VLDL and IDL
  - Modest ↓ LDL (however, in some patients with combined hyperlipidemias, ↑ LDL)
  - Increased HDL (most patients)
  - Used in hypertriglyceridemia
- Side effects: gallstones, myositis

Ezetimibe

- Mechanism: prevents intestinal absorption of cholesterol, results in ↓ LDL
- Side effect: gastrointestinal distress

Orlistat

- Therapeutic use: weight loss
- Mechanism: inhibits pancreatic lipase → ↓ triglyceride breakdown in the intestine
- Side effects: oily stools (steatorrhea), diarrhea; ↓ absorption of lipid-soluble vitamins

Cardiac and Renal Drug List and Practice Questions

7

Table III-7-1. The Major Cardiovascular and Renal Drugs

Antiarrhythmics	Antihypertensives	Antianginals
1A quinidine, procainamide	Thiazide diuretics	Nitrates: nitroglycerin, isosorbide
1B lidocaine	ACEIs: captopril, etc., and ARBs: losartan, etc. Renin inhibitor: aliskiren	CCBs: verapamil, nifedipine
1C flecainide	CCBs: verapamil, nifedipine, etc	β blockers: atenolol, etc.
II propranolol, acebutolol (ISA), esmolol	β blockers: atenolol, metoprolol, acebutolol, etc.	
III amiodarone, sotalol	α blockers: prazosin, doxazosin, etc.	
IV verapamil, diltiazem	α2 agonists: clonidine, methyldopa	
Adenosine	Vasodilators: hydralazine, nitroprusside, diazoxide, minoxidil Pulmonary hypertension: bosentan, epoprostenol, sildenafil	
Diuretics	**Drugs for Heart Failure**	**Antihyperlipidemics**
CA inhibitors: acetazolamide	ACEI or ARBs	Statins
Loops: ethacrynic acid, furosemide	Beta blockers	Resins: cholestyramine, colestipol
Thiazides: hydrochlorothiazide, indapamide, chlorthalidone	Diuretics	Other: nicotinic acid, ezetimibe, gemfibrozil, fenofibrate
K^+ sparing: amiloride, triamterene, spironolactone, eplerenone	Digoxin, bipyridines: inamrinone, milrinone; β agonists: dobutamine, dopamine	Weight loss: Orlistat

PRACTICE QUESTIONS

1. A patient has a genetic polymorphism such that they cannot rapidly metabolize drugs by acetylation. You would be most concerned about this polymorphism if the patient was taking which drug?

   A. Sotalol
   B. Clonidine
   C. Nitroglycerin
   D. Hydralazine
   E. Prazosin

2. Which side effect is associated with spironolactone?

   A. Alkalosis
   B. Hirsutism
   C. Hyperkalemia
   D. Hypercalcemia
   E. Hyperglycemia

3. Lidocaine is an effective antiarrhythmic because it

   A. suppresses excitability in hypoxic areas of the heart
   B. prolongs the QT interval
   C. prolongs the PR interval
   D. depresses the slope of phase 0 in slow response tissues
   E. acts on inhibitory G-protein coupled receptors

4. Sildenafil has been prescribed for years to treat erectile dysfunction. Recently, this drug is also being used for what condition?

   A. Vasospastic angina
   B. Supraventricular tachycardia
   C. Cyanide poisoning
   D. Raynaud disease
   E. Pulmonary hypertension

5. A patient with hypertension also suffers from essential tremor. Optimal treatment of the patient should include management with

   A. prazosin
   B. clonidine
   C. metoprolol
   D. lidocaine
   E. propranolol

6. Selective β-1 blockers are preferred over nonselective beta blockers in some patients because they
   - A. cause less cardiodepression
   - B. are less likely to cause bronchoconstriction
   - C. are more effective for migraine prophylaxis
   - D. are more effective as an antiarrhythmics
   - E. have greater prophylactic value post-MI

7. Which drug will utilize the same signaling pathway as endogenous bradykinin on smooth muscle?
   - A. minoxidil
   - B. nitroprusside
   - C. theophylline
   - D. phenylephrine
   - E. cocaine

8. A 75-year-old patient suffering from congestive heart failure accidentally ingests a toxic dose of digoxin. Clinical consequences due to the toxic effects of cardiac glycosides are likely to include
   - A. seizures
   - B. hypercalcemia
   - C. bicarbonaturia
   - D. intermittent claudication
   - E. visual disturbances

9. In the management of a cardiac arrhythmia, lidocaine is to be administered by way of an IV loading dose. What variable must be known to calculate an appropriate loading dose?
   - A. renal clearance
   - B. bioavailability
   - C. volume of distribution
   - D. lag time
   - E. time to steady-state

10. Both dobutamine and inamrinone increase cardiac contractility by
    - A. activation of adenylyl cyclase
    - B. inactivation of Na channels
    - C. inhibition of Na^+/K^+-ATPase
    - D. increasing cAMP
    - E. activation of Na/Cl cotransporter

11. Which one of the following is likely to occur following treatment of a hypercholesterolemic patient with cholestyramine?

    A. Increased recycling of bile salts
    B. Increased circulating cholesterol
    C. Decreased VLDL synthesis
    D. Downregulation of LDL receptors
    E. Elevation of plasma triglycerides

12. A new diuretic is being studied in human volunteers. Compared with placebo, the new drug increases urine volume, increases urinary Ca^{2+}, increases plasma pH, and decreases serum K^+. If this new drug has a similar mechanism of action to an established diuretic, it probably

    A. blocks the NaCl cotransporter in the DCT
    B. blocks aldosterone receptors in the CT
    C. inhibits carbonic anhydrase in the PCT
    D. inhibits the $Na^+/K^+/2Cl^-$ cotransporter in the TAL
    E. acts as an osmotic diuretic

13. Which one of the following drugs is most likely to block K+ channels in the heart responsible for cardiac repolarization, and also blocks calcium channels in the AV node?

    A. Amiodarone
    B. Quinidine
    C. Lidocaine
    D. Sotalol
    E. Verapamil

14. The treatment of hyperlipidemic patients with nicotinic acid (niacin) results in

    A. increases in VLDL
    B. decreases in both plasma cholesterol and TGs
    C. inhibition of HMG-CoA reductase
    D. decreases in HDL
    E. no change in total cholesterol in the plasma

15. Which drug is useful for patients with congestive heart failure because it reduces both preload and afterload, and also inhibits cardiac remodeling?

    A. Hydralazine
    B. Hydrochlorothiazide
    C. Prazosin
    D. Nifedipine
    E. Captopril

16. Enhancement of the effects of bradykinin is most likely to occur with drugs like

    A. clonidine
    B. diazoxide
    C. lisinopril
    D. losartan
    E. propranolol

17. Outpatient prophylaxis of a patient with an SVT is best accomplished with the administration of

    A. adenosine
    B. diltiazem
    C. esmolol
    D. lidocaine
    E. mexiletine

18. Which one of the following is the most appropriate drug to use for the patient described in parentheses?

    A. Captopril (60-year-old woman with diabetic nephropathy)
    B. Nitroprusside (50-year-old man with BP of 140/95 mm Hg)
    C. Losartan (29-year-old pregnant woman)
    D. Propranolol (40-year-old patient with peripheral vascular disease)
    E. Milrinone (57-year-old patient with chronic CHF)

19. In a patient suffering from angina of effort, nitroglycerin may be given sublingually because this mode of administration

    A. bypasses the coronary circulation
    B. causes less reflex tachycardia than oral administration
    C. improves patient compliance
    D. has a decreased tendency to cause methemoglobinemia
    E. avoids first-pass hepatic metabolism

20. A patient with a supraventricular tachycardia has an atrial rate of 280/min with a ventricular rate of 140/min via a 2:1 AV nodal transmission. After treatment with a drug, the atrial rate slowed to 180/min, but the ventricular rate increased to 180/min. Which of the following drugs was most likely to have been given to this patient?

    A. Adenosine
    B. Digoxin
    C. Esmolol
    D. Quinidine
    E. Verapamil

ANSWERS AND EXPLANATIONS

1. **Answer: D.** Hydralazine is metabolized by *N*-acetyltransferase (a phase II drug metabolism reaction) associated with a genetic polymorphisms. Patients who are classified as slow acetylators may develop SLE-like symptoms when treated with hydralazine. Other drugs metabolized via *N*-acetyltransferase, including isoniazid and procainamide, have also been associated with lupus-like symptoms in slow acetylators.

2. **Answer: C.** Spironolactone blocks aldosterone receptors thereby inhibiting the production of Na^+ channels in the collecting duct and is used as a K^+-sparing agent because the reabsorption of Na^+ in the CT is coupled (indirectly) to the secretion of K^+ ions. Hyperkalemia is characteristic of this drug and may lead to clinical consequences at high doses, or if patients fail to discontinue K^+ supplements or ingest foodstuffs high in K^+. Because Na^+ reabsorption is associated with secretion of protons, spironolactone causes retention of H^+ ions, leading to acidosis. It has no significant effect on the renal elimination of Ca^{2+} or on the plasma level; of glucose.

3. **Answer: A.** Lidocaine, a class IB drug, effectively targets ischemic areas of the heart. Its major effect is on sodium channels in fast response fibers such as ventricular muscle. It has no significant effect on the PR or QT intervals.

4. **Answer: E.** Sildenafil (a PDE5 inhibitor) is used for erectile dysfunction but has been recently approved for use in pulmonary hypertension. Other useful drugs in pulmonary hypertension are epoprostenol and bosentan.

5. **Answer: E.** Propranolol is a nonselective beta blocker useful in a variety of cardiac conditions including hypertension. The drug is also useful in essential tremor where blocking the beta-2 receptor is beneficial. Metoprolol, beta-1 selective, is useful in hypertension but not essential tremor. Clonidine and prazosin are second-line drugs for hypertension and not effective in essential tremor. Lidocaine, an antiarrhythmic, is not effective in either condition.

6. **Answer: B.** β1-selective blockers like atenolol and metoprolol are less likely to block receptors in the bronchiolar smooth muscle and therefore less likely to cause bronchoconstriction, especially in asthmatic patients. Nonselective beta blockers are considered to be equally as effective as selective beta-1 blockers in arrhythmias, migraine prevention, and in post-MI prophylaxis. Both types of drugs are cardiodepressant.

7. **Answer: B.** Bradykinin binds to endothelial receptors and causes the formation of nitric oxide, which signals through the cGMP pathway to relax smooth muscle. Nitroprusside utilizes nitric oxide and cGMP in a similar fashion to relax smooth muscle.

8. **Answer: E.** Digoxin toxicity is associated with CNS consequences including disorientation and visual dysfunctions such as halos around lights and blurry, yellow vision. More serious manifestations include life-threatening arrhythmias.

9. **Answer: C.** Back to basic principles! Recall that to calculate a loading dose you must know volume of distribution and target plasma concentration. Since lidocaine is being given IV, its bioavailability is 100% (f=1) so no adjustment is required to the equation. Renal clearance is needed to calculate a maintenance dose, and time to steady-state applies only when using a maintenance dose. There is no lag time for an IV drug.

10. **Answer: D.** Dobutamine acts as a beta-1 agonist to activate adenylyl cyclase and increase cAMP. Inamrinone inhibits phosphodiesterase III which increases the amount of cAMP in the heart. In each case, there is an increase in intracellular Ca^{2+} being sequestered in the SR which leads to enhance contractility.

11. **Answer: E.** Cholestyramine and colestipol are resins that sequester bile acids in the gut, preventing their reabsorption. This leads to release of their feedback inhibition of 7-alpha hydroxylase and the diversion of cholesterol toward new synthesis of bile acids. Increase in high-affinity LDL receptors on hepatocyte membranes decreases plasma LDL. These drugs have a small but significant effect to increase plasma HDL rather than decrease it, but their ability to increase TGs precludes their clinical use in the management of hypertriglyceridemias.

12. **Answer: D.** The effects described are typical of loop diuretics, which inhibit the $Na^+K^+2Cl^-$ cotransporter in the thick ascending limb. This action prevents the reabsorption of Ca^{2+} from the paracellular pathway and provides for the use of these drugs in hypercalcemia. The increased load of Na^+ in the collecting tubules leads to increased excretion of both K^+ and H^+, so hypokalemia and alkalosis may occur.

13. **Answer: A.** Amiodarone is a highly effective antiarrhythmic drug, in part because of its multiple actions, which include Na+ channel block, beta adrenoceptor block, K^+ channel block, and Ca^{2+} channel block. Drugs that block K^+ channels prolong APD and ERP and predispose toward torsades de pointes ventricular arrhythmias. Quinidine, class Ia, can block both sodium and potassium channels but not calcium channels. Lidocaine, class Ib, blocks only sodium channels. Sotalol is both a beta blocker and a potassium channel blocker. It is a class III drug that also has class II properties. Verapamil is a class IV calcium channel blocker with no effect on potassium.

14. **Answer: B.** Nicotinic acid inhibits the synthesis of the VLDL apoprotein and decreases VLDL production. Its use results in decreases of both cholesterol and triglycerides, so total cholesterol in the plasma decreases. The drug is not an inhibitor of HMG-CoA reductase, and it increases plasma HDL to a greater extent than any other available antihyperlipidemic drug.

15. **Answer: E.** Captopril and other ACE inhibitors are primary treatment options for congestive heart failure because they reduce preload by dilating veins and reduce afterload by dilating arterioles. They inhibit cardiac remodeling which results in improved survival for patients with heart failure. Hydralazine reduces afterload but does not affect preload. Hydrochlorothiazide reduces preload but does not affect afterload and does not inhibit remodeling. Neither prazosin nor nifedipine has any significant role in heart failure.

16. **Answer: C.** ACE inhibitors prevent the conversion of angiotensin I to angiotensin II and lower blood pressure by decreasing both the formation of aldosterone formation and the vasoconstrictive action of AII at AT-1 receptors. ACEIs also inhibit the metabolism of bradykinin, and this leads to additional hypotensive effects, because bradykinin is an endogenous vasodilator. Unfortunately, increases in bradykinin are associated with side effects, including cough and angioedema. Losartan, which blocks AT-1 receptors, does not increase bradykinin levels.

17. **Answer: B.** Supraventricular tachycardias (SVTs) are treated effectively by class II and class IV antiarrhythmics. In addition, adenosine is indicated for SVTs and nodal tachycardias but only acutely since it must be administered IV and has an extremely short duration. The primary actions of both beta blockers (esmolol) and CCBs (diltiazem) are at the AV node, but esmolol is too short-acting to be useful as prophylaxis. Lidocaine and mexiletine are both class Ib drugs that are used in ventricular arrhythmias.

18. **Answer: A.** ACEIs slow the progression of diabetic nephropathy and are indicated for management of HTN in such patients. Nitroprusside is used IV in severe HTN or hypertensive crisis, not for management of mild-to-moderate HTN. Losartan, which blocks AT-1 receptors, is associated with teratogenic effects during fetal development, as are the ACEIs. Nonselective beta blockers are not ideal for patients who suffer from peripheral vascular disease, diabetes, or asthma. Milrinone, like most inotropes, is not useful long-term in CHF patients. The drug has been shown to increase mortality with chronic use, and thus is indicated for acute CHF. Digoxin is currently the only inotrope used chronically.

19. **Answer: E.** The sublingual administration of a drug avoids its absorption into the portal circulation and hence eliminates the possibility of first-pass metabolism, which can often have a major impact on oral bioavailability. Given sublingually, nitroglycerin is more effectively absorbed into the systemic circulation and has improved effectiveness in angina by this mode of administration. Effective absorption is unlikely to decrease reflex tachycardia or propensity toward methemoglobinemia. There is no bypass of the coronary circulation—nitrates actually decrease coronary vasospasm, which makes them effective in variant angina.

20. **Answer: D.** An increase in AV conduction is characteristic of quinidine, which exerts quite marked blocking actions on muscarinic receptors in the heart. Thus, an atrial rate, formerly transmitted to the ventricles in a 2:1 ratio, may be transmitted in a 1:1 ratio after quinidine. This effect of quinidine can be offset by the prior administration of an antiarrhythmic drug that decreases AV nodal conduction, such as digoxin or verapamil. All of the drugs listed (except quinidine) slow AV nodal conduction, but adenosine and esmolol (a beta blocker) are very short-acting agents used IV only.

PART IV

CNS Pharmacology

1 Sedative-Hypnotic-Anxiolytic Drugs

Learning Objectives

- ❑ Answer questions related to benzodiazepines and barbiturates

DRUGS USED FOR SEDATION, SLEEP, ANXIETY

Drugs used for sedation, sleep, and anxiety can cause dose-dependent CNS depression that extends from sedation to anesthesia to respiratory depression and even death. Benzodiazepines (BZs) reach a plateau in CNS depression, while barbiturates and alcohol do not.

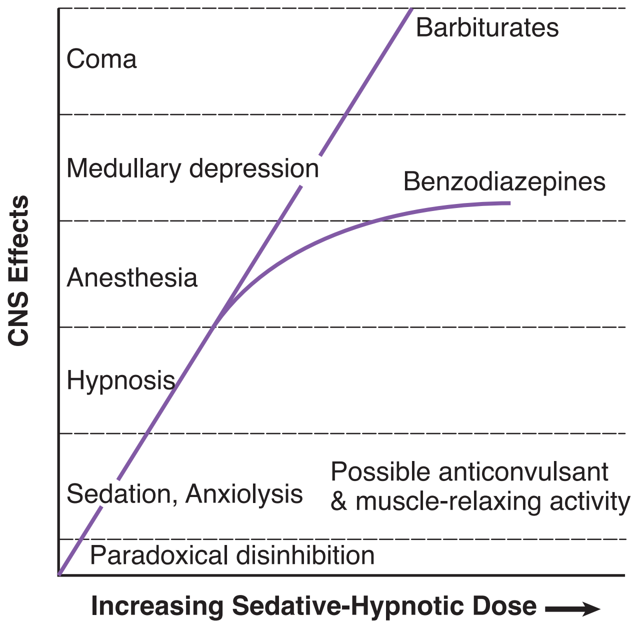


Figure IV-1-1. CNS Effects Associated with Increasing Doses of Sedative-Hypnotic (S-H) Drugs

Mechanisms

High-Yield

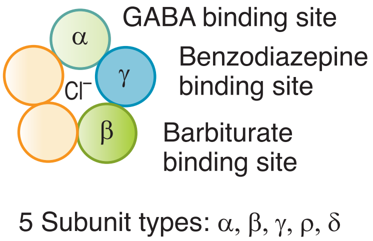


Figure IV-1-2. Site of Action of Drugs on the $GABA_A$ Complex

- $GABA_A$ activation ↑ Cl^- influx
- $GABA_B$ activation ↑ K^+ efflux
- Both mechanisms result in membrane hyperpolarization
  - BZs:
    - Potentiate GABA
    - ↑ the frequency of Cl– channel opening
    - Have no GABA mimetic activity
    - Act through BZ receptors
    - These receptors are part of the $GABA_A$ complex
    - BZ_1 mediates sedation
    - BZ_2 mediates antianxiety and impairment of cognitive functions
  - Barbiturates:
    - Prolong GABA activity
    - ↑ duration of Cl– channel opening
    - Have GABA mimetic activity at high doses
    - Do not act through BZ receptors
    - Have their own binding sites on the $GABA_A$ complex
    - Also inhibit complex I of electron transport chain

Clinical Correlate

Flumazenil

This nonspecific BZ receptor antagonist is used to reverse the CNS depression caused by BZs used in anesthesia or in BZ overdose. Flumazenil cannot reverse the CNS depression caused by barbiturates and alcohols.

Uses of Benzodiazepines

High-Yield

Table IV-1-1. Uses of Various Benzodiazepines

Drug	Indications
Alprazolam	Anxiety, panic, phobias
Diazepam	Anxiety, preop sedation, muscle relaxation, withdrawal states
Lorazepam	Anxiety, preop sedation, status epilepticus (IV)
Midazolam	Preop sedation, anesthesia IV
Temazepam	Sleep disorders
Oxazepam	Sleep disorders, anxiety

Other Properties

- Pharmacokinetics of BZs: liver metabolites are also active compounds, except for oxazepam, temazepam, and lorazepam
- Uses of barbiturates: phenobarbital for seizures
- Pharmacokinetics of barbiturates:
  - Liver metabolized, sometimes to active compounds
  - General inducers of cytochrome P450s
  - Contraindication in porphyrias
- Tolerance to and dependence on sedative-hypnotics:
  - Chronic use leads to tolerance
  - Cross-tolerance occurs between BZs, barbiturates, and ethanol
  - Psychologic and physical dependence occur
  - But abuse liability of BZs is < ethanol or barbiturates
  - Withdrawal signs of BZs:
    - Rebound insomnia
    - Anxiety
    - Seizures when BZs were used as antiepileptic or in high doses
  - Withdrawal signs of barbiturates and ethanol: anxiety, agitation, life-threatening seizures (delirium tremens with alcohol)
  - Management of withdrawal: supportive and long-acting BZs
- Drug interactions: $GABA_A$ drugs are:
  - Additive with other CNS depressants (possible life-threatening respiratory depression), such as anesthetics, antihistamines, opiates, β-blockers, etc.
  - Barbiturates induce metabolism of most lipid-soluble drugs, such as oral contraceptives, carbamazepine, phenytoin, warfarin, etc.

Non-BZ Drugs

- Zolpidem and zaleplon
  - BZ_1 receptor agonist
  - Less effect on cognitive function (BZ_2-mediated)
  - Overdose reversed by flumazenil
  - Used in sleep disorders
  - Less tolerance and abuse liability (sleepwalking)
- Buspirone
  - No effect on GABA
  - 5-HT_{1A} partial agonist
  - Used for generalized anxiety disorders
  - Nonsedative
  - Takes 1 to 2 weeks for effects

Alcohols 2

Learning Objectives

- ❏ Answer questions about the mechanism of action and metabolism of alcohol

All alcohols cause CNS depression, in part through GABA mimetic activity. All alcohols cause metabolic acidosis.

Clinical Correlate

Alcohol and Pregnancy

Fetal alcohol syndrome is characterized by growth restriction, midfacial hypoplasia, microcephaly, and marked CNS dysfunction, including the frequent occurrence of mental retardation.

Note

Drugs that cause disulfiram-like effects:

- Metronidazole
- Griseofulvin

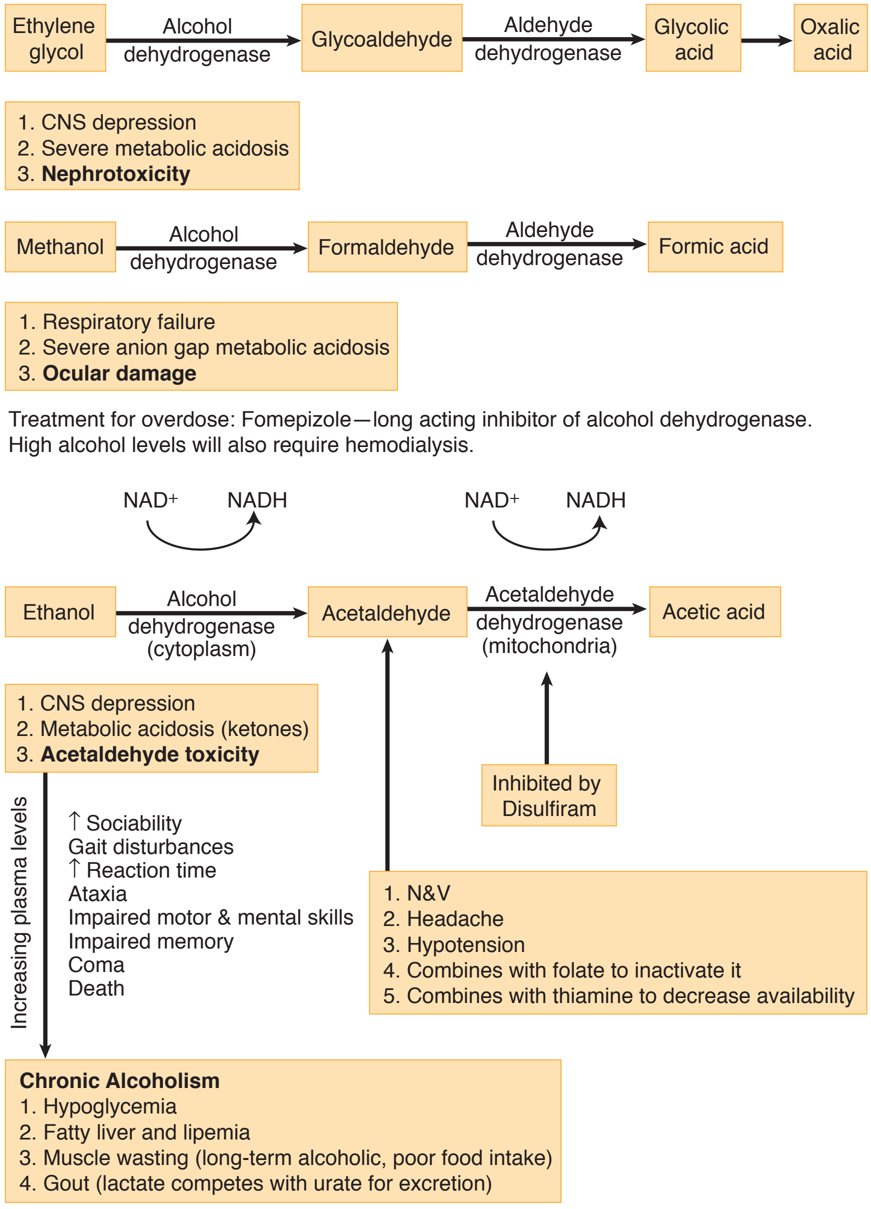


Figure IV-2-1. Metabolism and Pharmacologic Actions of the Alcohols

Drugs Used for Depression, Bipolar Disorders, and Attention Deficit Hyperactivity Disorder (ADHD)

3

Learning Objectives

- ❏ Explain information related to drugs used in depression bipolar disorders, and ADHD
- ❏ Solve problems related to the use of lithium

- "Amine hypothesis" of depression:
  - Reserpine: depletes NE, 5HT, DA, and causes severe depression
  - Acute mechanism of antidepressants: ↑ NE, ↑ 5HT
  - However, antidepressant effect takes several weeks to occur.

DRUGS USED IN DEPRESSION

Selective Serotonin Reuptake Inhibitors (SSRIs)

High-Yield

- Drugs: **fluoxetine, paroxetine, sertraline, citalopram, fluvoxamine**
- Mechanism: selective blockade of 5HT reuptake
- Uses:
  - Major depression
  - OCD
  - Bulimia
  - Anxiety disorders (chronic treatment/acute, benzodiazepines)
  - Premenstrual dysphoric disorder (PMDD)
- Side effects: anxiety, agitation, bruxism, sexual dysfunction, weight loss
- Toxicity: serotonin syndrome
- Drug interactions
  - ↑ 5HT: serotonin syndrome
    - Symptoms: sweating, rigidity, myoclonus, hyperthermia, ANS instability, seizures
    - Drugs: MAOIs, TCAs, and meperidine

  - Most inhibit cytochrome P450 enzymes (in particular, fluvoxamine and fluoxetine)
  - Important interaction includes increased levels of benzodiazepines in treatment of anxiety disorders
  - Citalopram is safer for interactions

Tricyclic Antidepressants (TCAs)

High-Yield

- Drugs: **amitriptyline, imipramine,** and **clomipramine**
- Mechanism: nonspecific blockade of 5HT and NE reuptake
- Uses:
  - Major depressions
  - Phobic and panic anxiety states
  - Obsessive-compulsive disorders (OCDs)
  - Neuropathic pain
  - Enuresis
- Side effects: muscarinic and α blockade
- Toxicity: the "3 Cs": coma, convulsions, and cardiotoxicity
- Drug interactions:
  - Hypertensive crisis with MAO inhibitors
  - Serotonin syndrome with SSRIs, MAO inhibitors, and meperidine
  - Prevent antihypertensive action of α_2 agonists

MAO Inhibitors

- Drugs: **phenelzine** and **tranylcypromine**
- Mechanism: irreversible inhibition of MAO_A and MAO_B
- Use: atypical depressions
- Drug interactions
  - Serotonin syndrome: SSRIs, TCAs, and meperidine
  - ↑ NE: hypertensive crisis
    - Symptoms: ↑ BP, arrhythmias, excitation, hyperthermia
    - Drugs: releasers (i.e., tyramine), tricyclic antidepressants (TCAs), α_1 agonists, levodopa

Other Antidepressants

High-Yield

- Trazodone: associated with cardiac arrhythmias and priapism
- Venlafaxine: nonselective reuptake blocker devoid of ANS side effects
- **Bupropion:** dopamine reuptake blocker; used in smoking cessation
- Mirtazapine: α_2 antagonist, associated with weight gain

Clinical Correlate

Varenicline is a partial agonist of nicotinic receptors and is used in smoking cessation.

DRUGS USED IN BIPOLAR DISORDERS

Lithium

High-Yield

Lithium remains DOC for bipolar disorders. Antidepressants/antipsychotics are also usually required.

- Mechanism:
  - Prevents recycling of inositol (↓ PIP_2) by blocking inositol monophosphatase
  - ↓ cAMP
- Side effects:
  - Narrow therapeutic index; requires therapeutic monitoring
  - Tremor, flu-like symptoms, life-threatening seizures
  - Hypothyroidism with goiter (↓ TSH effects and inhibition of 5′-deiodinase)
  - Nephrogenic diabetes insipidus (↓ ADH effect); manage with amiloride
- Teratogenicity: Ebstein's anomaly (malformed tricuspid valve)

Other drugs used in bipolar disorders include valproic acid and carbamazepine.

DRUGS USED IN ADHD

Methylphenidate

- Amphetamine-like
  - Side effects: agitation, restlessness, insomnia, cardiovascular toxicity

Atomoxetine

- Selective NE reuptake inhibitor
  - Side effects: *See* TCA section, above.

Recall Question

A drug that can cause serotonin syndrome is:

A. Bupropion

B. Lithium

C. Fluoxetine

D. Trazodone

Answer: C

Drugs Used in Parkinson Disease and Psychosis

4

Learning Objectives

- ❑ Answer questions about dopaminergic neural pathways
- ❑ Demonstrate understanding of dopamine receptors
- ❑ Compare and contrast the mechanism of action and side-effects for drugs used in Parkinson disease with antipsychotic drugs

DOPAMINERGIC NEURAL PATHWAYS

In the CNS, dopamine (DA) is a precursor to NE in diffuse noradrenergic pathways and is an inhibitory neurotransmitter in the major dopaminergic pathways.

Nigrostriatal Tract

- Cell bodies in the substantia nigra project to the striatum, where they release DA, which inhibits GABA-ergic neurons. In Parkinson disease, the loss of DA neurons in this tract leads to excessive ACh activity → extrapyramidal dysfunction.
- DA receptor antagonists → pseudo-Parkinsonism (reversible).
- DA agonists may cause dyskinesias.

Mesolimbic-Mesocortical Tract

- Cell bodies in midbrain project to cerebrocortical and limbic structures
- Functions include regulation of affect, reinforcement, cognitive functions, and sensory perception. Psychotic disorders and addiction are partly explained by ↑ DA in these pathways.
- Drugs that ↑ DA functions →↑ reinforcement and, at high doses, may cause psychoses.
- DA antagonists →↓ cognitive function

Tuberoinfundibular

- Cell bodies in hypothalamus project to anterior pituitary and release DA →↓ prolactin.
- DA agonists are used in hyperprolactinemic states.
- DA antagonists may cause endocrine dysfunction, including gynecomastia and amenorrhea/galactorrhea.

Chemoreceptor Trigger Zone

- Activation of DA receptors →↑ emesis.
- DA agonists (e.g., apomorphine) are emetic, and DA antagonists are antiemetic.

DOPAMINE RECEPTORS

- D_1-like: G_s coupled
- D_2-like: G_i coupled
  - D_{2A}: nigrostriatal
  - D_{2C}: mesolimbic

DRUGS USED IN PARKINSON DISEASE

Signs and symptoms of Parkinson disease include bradykinesia, muscle rigidity, and resting tremor.

The pathology of Parkinson involves the degeneration of nigrostriatal dopamine tracts with imbalance between dopamine (decreased) and ACh (increased).

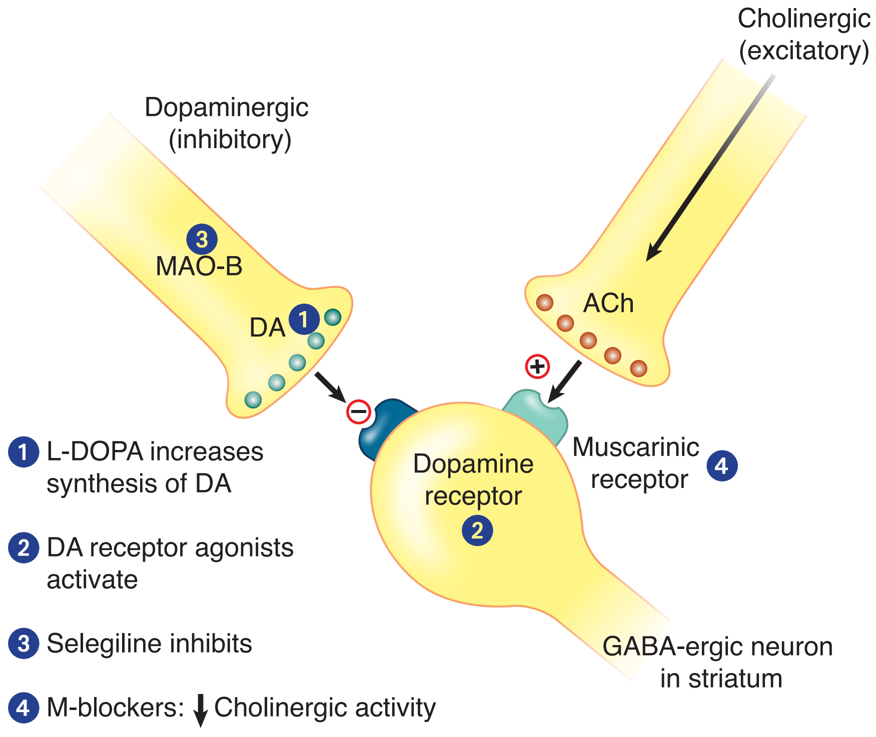


Figure IV-4-1. CNS Targets for Antiparkinsonian Drugs

The pharmacologic strategy for Parkinson disease is to restore normal dopamine and decrease ACh activity at muscarinic receptors in the striatum.

Drugs Increasing Dopamine Function

High-Yield

- **Levodopa**
  - Prodrug converted to dopamine by aromatic amino acid decarboxylase (AAAD)
  - Given with carbidopa
  - Side effects: dyskinesias, "on-off" effects, psychosis, hypotension, vomiting

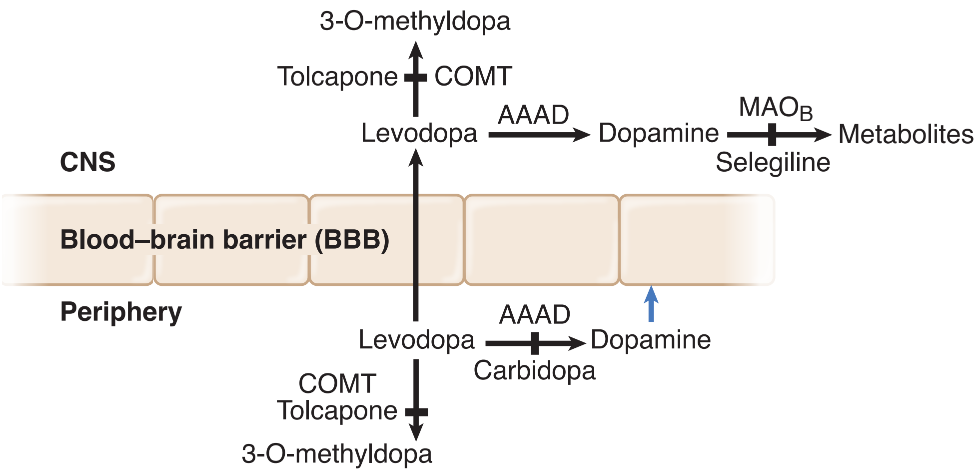


Figure IV-4-2. Inhibitors of Levodopa Metabolism

- **Tolcapone** and **entacapone**
  - COMT converts L-dopa to 3-O-methyldopa, a partial agonist at dopamine receptors.
  - These drugs inhibit COMT and enhance levodopa uptake and efficacy.
  - Tolcapone is hepatotoxic.
- **Selegiline**
  - MAO_B-selective inhibitor (no tyramine interactions)
  - Initial treatment and adjunct to levodopa
  - Side effects: dyskinesias, psychosis, insomnia (metabolized to amphetamine)

Dopamine-Receptor Agonists

High-Yield

- **Pramipexole** and **ropinirole**
- **Bromocriptine**
  - Use: hyperprolactinemia and acromegaly
- Side effects: dyskinesias and psychosis

Drugs Decreasing ACh Function

- **Benztropine** and **trihexyphenidyl**, which are muscarinic blockers
  - Actions: ↓ tremor and rigidity but have little effects on bradykinesia
  - Side effects: atropine-like
- **Amantadine**
  - Antiviral, which block muscarinic receptors and ↑ dopamine release
  - Side effects: atropine-like and livedo reticularis

ANTIPSYCHOTIC DRUGS

Schizophrenia

Positive symptoms of schizophrenia include thought disorders, delusions, hallucinations, and paranoia. Negative symptoms include amotivation, social withdrawal, flat affect, and poverty of speech.

The "dopamine hypothesis" of schizophrenia states that symptoms arise because of excessive dopaminergic activity in mesolimbic system. Dopamine agonists cause psychosis. Dopamine antagonists have antipsychotic actions.

Serotonin is increasingly seen as a part of the etiology of schizophrenia.

Mechanisms

High-Yield

- Blockade of dopamine receptors
- Blockade of $5HT_2$ receptors

Uses

- Schizophrenia
- Schizoaffective states
- Bipolar disorder
- Tourette syndrome and Huntington disease
- Drug or radiation emesis

Side Effects

High-Yield

- From dopamine blockade
  - Dyskinesias (extrapyramidal symptoms [EPS])
    - **Acute EPS:** pseudoparkinsonism, dystonia, akathisia (management: antimuscarinic drugs [benztropine or diphenhydramine])
    - **Chronic EPS:** tardive dyskinesia (TD) (management: discontinuation/switch to atypical)
  - Dysphoria
  - Endocrine dysfunction
    - Temperature regulation problems (neuroleptic malignant syndrome [NMS], treated with dantrolene and bromocriptine) (see chapter 6)
    - Increased prolactin (galactorrhea, amenorrhea, gynecomastia)
    - Eating disorders (weight gain)
- From muscarinic blockade (particularly tachycardia and decreased seizure threshold)
- From alpha blockade (particularly hypotension)

Table IV-4-1. Characteristic Properties of Antipsychotic Drugs

Drug Group Examples	EPS*	M Block	Sedation	Alpha Block	Other Characteristics
Typicals					
Chlorpromazine	++	++	+++	+++	NA
Thioridazine	+	+++	+++	+++	• Cardiotoxicity (torsades—"quinidine-like") • Retinal deposits
Fluphenazine	+++	+	+	+	NA
Haloperidol	+++	+	+	+	Most likely cause of neuroleptic malignant syndrome (NMS) and TD
Atypicals					
Clozapine	+/–	++	+	+++	• Blocks D_{2c} and $5HT_2$ receptors • No TD • Agranulocytosis—(weekly WBC count) requirement for weekly blood test, weight gain • Increased salivation ("wet pillow"syndrome) • Seizures
Olanzapine	+/–	+	+	++	Blocks $5HT_2$ receptors, improves negative-symptoms
Risperidone	+	+/–	++	++	Blocks $5HT_2$ receptors, improves negative symptoms
Aripiprazole	+	+/–	+/–	+/–	Partial agonist of D_2 receptor; blocks $5HT_2$ receptors
Other atypicals: quetiapine, ziprasidone					

* Extrapyramidal symptoms

Clinical Correlate

Parenteral formulations of certain antipsychotic drugs (e.g., fluphenazine, haloperidol) are available for rapid initiation of treatment and for maintenance therapy in noncompliant patients. Depot forms of both drugs exist.

Anticonvulsants 5

Learning Objectives

- ❏ Describe the mechanism of action and unique features of the commonly used anticonvulsants
- ❏ Provide an overview of which anticonvulsants are used for which types of seizures

DRUGS USED IN SEIZURES

Seizures result from episodic electrical discharges in cerebral neurons associated with prolonged depolarization, during which sustained, high-frequency, repetitive firing (SHFRF) occurs, followed by prolonged hyperpolarization. The goal of drug management is restoration of normal patterns of electrical activity.

Mechanisms of Action

High-Yield

- Decreased axonal conduction by preventing Na^+ influx through fast Na channels: carbamazepine, phenytoin
- Increased inhibitory tone by facilitation of GABA-mediated hyperpolarization: barbiturates, benzodiazepines
- Decreased excitatory effects of glutamic acid: lamotrigine, topiramate (blocks AMPA receptors); felbamate (blocks NMDA receptors)
- Decreased presynaptic Ca^{2+} influx through type-T channels in thalamic neurons: ethosuximide and valproic acid

Table IV-5-1. Seizure States and Effective Drugs

Seizure Type	Effective Drugs
Partial—simple or complex	Valproic acid, phenytoin, carbamazepine, lamotrigine
General—tonic-clonic	Valproic acid, phenytoin, carbamazepine, lamotrigine
General—absence	Ethosuximide, valproic acid
Status epilepticus	Lorazepam, diazepam, phenytoin, or fosphenytoin*

* IV fosphenytoin is more water soluble.

Primary Anticonvulsants

High-Yield

- **Phenytoin**
  - Blocks axonal Na^+ channels in their inactivated state
  - Prevents seizure propagation
  - Uses: seizure states
  - Pharmacokinetics:
    - Variable absorption
    - Nonlinear kinetics
    - Induction of cytochrome P450s
    - Zero-order kinetic of elimination
  - Side effects:
    - CNS depression
    - Gingival hyperplasia
    - Hirsutism
    - Osteomalacia (↓ vitamin D)
    - Megaloblastic anemia (↓ folate)
    - Aplastic anemia (check hematology lab results)
  - Teratogenicity: cleft lip and palate
- **Carbamazepine**
  - Mechanism identical to phenytoin
  - Uses:
    - Seizure states
    - DOC for trigeminal neuralgia
    - Bipolar disorder
  - Pharmacokinetics: induces cytochrome P450, including its own metabolism
  - Side effects:
    - CNS depression
    - Osteomalacia
    - Megaloblastic anemia
    - Aplastic anemia
    - Exfoliative dermatitis
    - ↑ ADH secretion (dilutional hyponatremia)
  - Teratogenicity: cleft lip and palate; spina bifida

- **Valproic acid**
  - Mechanism:
    - Similar to phenytoin
    - But also inhibition of GABA transaminase
    - Blockade of T-type Ca^{2+} channels
  - Uses:
    - Seizure states
    - Mania of bipolar disorders
    - Migraine prophylaxis
  - Pharmacokinetics: inhibits cytochrome P450s
  - Side effects:
    - Hepatotoxicity (from toxic metabolite)
    - Thrombocytopenia
    - Pancreatitis
    - Alopecia
  - Teratogenicity: spina bifida
- **Ethosuximide**
  - Mechanism: blockade of T-type Ca^{2+} channels in thalamic neurons
  - Use: absence seizures

Other Anticonvulsants

- **Lamotrigine**
  - Blocks Na^{+} channels and glutamate receptors
  - Used in various seizures
  - Side effects: Stevens-Johnson syndrome
- **Levetiracetam**
  - Mechanism unclear
  - Used in focal-onset and generalized tonic-clonic seizures
- **Topiramate**
  - Blocks Na^{+} channels and glutamate receptors and enhances GABA activity
  - Used in focal seizures in adults and children > age 2; also used in migraine prophylaxis
  - Side effects: weight loss

- **Felbamate**
  - Block Na^+ channels and glutamate receptors
  - Used in seizure states (often adjunct therapy)
  - Side effects: Aplastic anemia
- **Gabapentin**
  - May affect calcium channels and neurotransmitter release, GABA effects
  - Used in seizure states, neuropathic pain (such as postherpetic neuralgia)

General features of anticonvulsant drug use include:

- Anticonvulsants are additive with other CNS depressants
- Avoid abrupt withdrawal, which may precipitate seizures
- ↓ efficacy of oral contraceptives via induction of cytochrome P450

Drugs Used in Anesthesia 6

Learning Objectives

- ❑ Demonstrate understanding of general anesthetics
- ❑ Explain information related to local anesthetics
- ❑ Use knowledge of skeletal muscle relaxants to solve problems

GENERAL ANESTHETICS

Inhaled Anesthetics

High-Yield

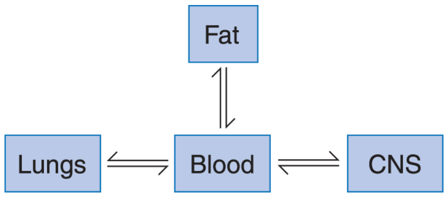


Figure IV-6-1. Compartmentalization of Anesthetics in the Body

Table IV-6-1. Properties of Specific Inhaled Anesthetics

Anesthetic	MAC Value	Blood–Gas Ratio	CV Effects	Specific Characteristics
Nitrous oxide	104%	0.5	Minimal	Rapid onset and recovery, no metabolism Diffusional hypoxia Spontaneous abortions
Sevoflurane	2%	0.6	Minimal	
Desflurane	6%	0.5	Minimal	

Anesthesia protocols include several agents in combinations.

- Inhaled anesthetics have varying potency in proportion to their lipid solubility.
- A MAC (minimal alveolar anesthetic concentration) is defined as the concentration of inhaled anesthetic (as a % of inspired air) at which 50% of patients do not respond to a surgical stimulus.

  - MAC is a measure of potency: ED50.
  - The more lipid soluble the anesthetic, the lower the MAC and the greater the potency.
  - MAC values are additive.
  - MAC values are lower in the elderly and in the presence of opiates or sedative-hypnotics.
- Rates of onset and recovery depend on the blood–gas ratio:
  - The more soluble the anesthetic in the blood, the slower the anesthesia.
  - Anesthetics with high blood–gas ratios are associated with slow onset.
  - Anesthetics with high blood–gas ratios are associated with slow recovery.
  - Anesthetics with low blood–gas ratios have fast onset and recovery.

Intravenous Anesthetics

- Midazolam
  - Benzodiazepine used for:
    - Preoperative sedation
    - Anterograde amnesia
    - Induction
    - Outpatient surgery
  - Depresses respiratory function
- Propofol
  - Used for induction and maintenance of anesthesia
  - Antiemetic
  - CNS and cardiac depressant
- Fentanyl
  - Opiate used for induction and maintenance of anesthesia
  - Depresses respiratory function
  - *See* Opioid Analgesics, chapter 7 in this section
- Ketamine
  - Dissociative anesthetic
  - NMDA-receptor antagonist
  - Induction of anesthesia
  - Emergent delirium, hallucinations
  - Cardiovascular stimulation
  - ↑ intracranial pressure

LOCAL ANESTHETICS

Local anesthetics provide regional anesthesia. Drugs come in 2 types. **Esters** include procaine, cocaine, benzocaine, which are metabolized by plasma and tissue esterases. **Amides** include lidocaine, bupivacaine, mepivacaine which metabolized by liver amidases.

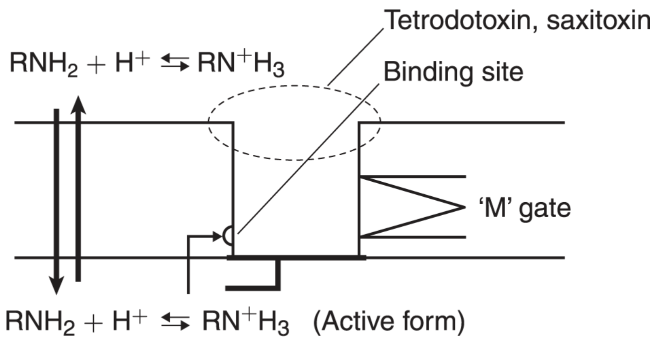


Figure IV-6-2. Mode of Action of Local Anesthetics

Mechanisms

The nonionized form of the drug crosses the axonal membrane. Once inside the nerve, the ionized form blocks the inactivated Na^+ channel. The drug slows recovery and prevents propagation of action potentials.

- Nerve fiber sensitivity:
  - Nerve fibers most sensitive to blockade are of smaller diameter and have high firing rates
  - The order of sensitivity is:

    type B and C > type A_δ > type A_β and A_γ > type A_α
  - Recovery is in reverse order
- Absorption:
  - Coadministration of α_1 agonists:
    - ↓ local anesthetic absorption into the systemic circulation
    - Prolong effects and ↓ toxicity

Side Effects

- Neurotoxicity
- Cardiovascular toxicity
- Allergies (esters via PABA formation)

Note

Esters and Amides

Local anesthetics that are esters have just one "i" in their names (e.g., procaine, cocaine); amide local anesthetics have more than one "i" (e.g., lidocaine, bupivacaine).

Note

Na^+ Channel Toxins

- Tetrodotoxin (from puffer fish) and saxitoxin (algae toxin, "red tide")
  - Block activated Na^+ channels
  - ↓ Na^+ influx
- Ciguatoxin (exotic fish) and batrachotoxin (frogs)
  - Bind to activated Na^+ channels
  - Cause inactivation
  - Prolong Na^+ influx

Note

Cocaine intrinsically causes vasoconstriction by blocking norepinephrine uptake.

SKELETAL MUSCLE RELAXANTS

Nicotinic receptors have 5 subunits. Two ACh bind each to 2 α subunits in order to open the Na^+ channel. This depolarizes the muscle.

Figure IV-6-3. Nicotinic ACh Receptor of the Neuromuscular Junction

Muscle relaxants are used mainly in anesthesia protocols or in the ICU to afford muscle relaxation and/or immobility. They interact with nicotinic ACh receptors at the neuromuscular junction.

Drugs

High-Yield

- **Nondepolarizing (competitive)**
  - Nicotinic antagonists
  - Rocuronium is the prototype
  - Reversible with AChE inhibitors
  - Progressive paralysis (face, limbs, respiratory muscle)
  - No effects on cardiac and smooth muscle
  - No CNS effects
  - Specific drugs: **atracurium** (rapid recovery, safe in hepatic or renal impairment, spontaneous inactivation to laudanosine (laudanosine can cause seizures)
- **Depolarizing (noncompetitive)**
  - Nicotinic agonist
  - Specific drug: **succinylcholine**
  - Two phases: **phase I** is depolarization, fasciculation, prolong depolarization, flaccid paralysis; **phase II** is desensitization
  - AChE inhibitors ↑ phase I; may reverse phase II
  - Rapidly hydrolyzed by pseudocholinesterase: short duration
  - Cautions: atypical pseudocholinesterase; hyperkalemia; malignant hyperthermia

Centrally Acting Skeletal Muscle Relaxants

- Benzodiazepines through $GABA_A$ receptors
- Baclofen through $GABA_B$ receptors
- Use: spasticity

Bridge to Pathology/Genetics

Malignant Hyperthermia

A life-threatening syndrome characterized by muscle rigidity, hyperthermia, hypertension, acidosis, and hyperkalemia.

- Associated with the use of skeletal muscle relaxants, especially succinylcholine, used in anesthesia regimens
- Genotypic susceptibility may be related to mutations in the genes, encoding ryanodine receptors and/or a protein component of L-type calcium channel in skeletal muscle

Treatment

Dantrolene acts directly on skeletal muscle to decrease contractility by blocking Ca^{2+} release from the sarcoplasmic reticulum. It is used in states that include extreme muscle rigidity, such as malignant hyperthermia associated with inhaled anesthetics and skeletal muscle relaxants or neuroleptic malignant syndrome associated with antipsychotics.

Opioid Analgesics

7

Learning Objectives

- ❑ Describe the site of action, effects, and common complications associated with morphine use
- ❑ Differentiate between mu-receptor agonists, antagonist, and mixed agonist-antagonist
- ❑ Describe the appropriate use of these medications in the treatment of pain, opiate withdrawal, and drug abuse

PROPERTIES OF OPIOIDS

Opioid analgesics are endogenous opiate peptides represented by endorphins, enkephalins, and dynorphins. There are 3 receptor families: μ, κ, and δ.

Presynaptic and postsynaptic inhibition occurs through G_i coupling. **Mu pharmacology** is the most important. Morphine is the prototype μ-agonist.

Morphine

High-Yield

- Analgesia: ↑ pain tolerance and ↓ perception and reaction to pain
- Sedation
- Respiratory depression: ↓ response to ↑ pCO_2 (do not give O_2; give naloxone)
- Cardiovascular: minimal effects on heart but vasodilation (avoid in head trauma)
- Smooth muscle: longitudinal relaxes; circular constricts
  - GI: ↓ peristalsis, constipation, cramping
  - GU: urinary retention, urgency to void
  - Biliary: ↑ pressure
  - Pupils: miosis
- Cough suppression: antitussive action, independent of analgesia and respiratory depression
- Nausea and vomiting: stimulation of the chemoreceptor trigger zone (CTZ) in the area postrema
- Increased histamine release
- Pharmacokinetics: glucuronidation; morphine-6-glucuronide is highly active; caution in renal dysfunction

Clinical Correlate

Contraindications for Opioids

- Head injury (possible increased intracranial pressure)
- Pulmonary dysfunction (except pulmonary edema)
- Hepatic/renal dysfunction (possible accumulation)
- Adrenal or thyroid deficiencies (exaggerated responses)
- Pregnancy (possible neonatal depression or dependence), except meperidine which does not inhibit uterine contractions in delivery and causes less respiratory depression in newborn

Clinical Correlate

Seizures caused by meperidine cannot be treated with opioid antagonists; use benzodiazepines.

Other Opioids and Analgesics

High-Yield

Table IV-7-1. Other Opioids and Analgesics

Receptor Action	Drug	Characteristics
Full agonists	Meperidine Methadone Codeine	• Also antimuscarinic No miosis Tachycardia No spasm GI/GU/gallbladder • Metabolized by cytochrome P450 to normeperidine, a serotonin reuptake inhibitor; normeperidine may cause serotonin syndrome and seizures • Used in maintenance of opiate addict • Cough suppressant • Analgesia • Used in combination with NSAIDs
Partial Agonist	**Buprenorphine**	**Precipitation of Withdrawal**
Mixed agonist-antagonists	Nalbuphine, pentazocine	• κ agonist spinal analgesia dysphoria • μ antagonist precipitation of withdrawal
Antagonists	Naloxone Naltrexone Methylnaltrexone	• IV, reversal for respiratory depression • PO, ↓ craving for alcohol and used in opiate addiction • Treatment of opioid-induced constipation (does not cross BBB and won't precipitate withdrawal)

Other Features of Opioid Analgesics

- Side effects of opioid analgesics:
  - Acute toxicity: classic triad
    - Pinpoint pupils
    - Respiratory depression
    - Coma
  - Management of acute toxicity: supportive, IV naloxone

- Abuse liability of opioid analgesics:
  - Tolerance: pharmacodynamic; occurs to all effects, except miosis and constipation
  - Dependence: physical and psychologic
  - Withdrawal: y
    - Yawning
    - Lacrimation, rhinorrhea, salivation
    - Anxiety, sweating, goose bumps
    - Muscle cramps, spasms, CNS-originating pain
  - Management of withdrawal: supportive, methadone, clonidine
- Opiate-related drugs with specific indications
  - Loperamide: diarrhea
  - Dextromethorphan: cough

Drugs of Abuse

8

Learning Objectives

- ❑ Provide an overview of the main classes of medications that are abused and controlled
- ❑ Give examples of drugs in each class and describe their effect, toxicity, and withdrawal response

Table IV-8-1. Properties of Drugs of Abuse

CNS Stimulants	Cocaine	Amphetamines
Neurotransmitters involved	NE, DA, 5HT	
Mechanism(s) of action	Blocks DA, NE, and 5HT reuptake in CNS; local anesthetic action from Na^+ channel blockade	Blockade of reuptake of NE and DA, release amines from mobile pool, weak MAO inhibitors
Effects	1. Increase NE: sympathomimetic effect with increased heart rate and contractility, blood pressure changes, mydriasis, and central excitation, hyperactivity 2. Increase DA: psychotic episodes, paranoia, hallucinations, possible dyskinesias, and endocrine disturbances 3. Increase 5HT: behavioral changes, aggressiveness, dyskinesias, and decreased appetite	
Toxicity	1. Excess NE: cardiac arrhythmias, generalized ischemia with possible MI and strokes; acute renal and hepatic failures 2. Excess DA: major psychosis, cocaine delirium 3. Excess 5HT: possible serotonin syndrome 4. All of the above: convulsion, hyperpyrexia, and death	
Withdrawal	Craving, severe depression, anhedonia, anxiety; manage with antidepressants	
CNS Depressants	**Benzodiazepines**	**Barbiturates and Ethanol**
Neurotransmitters involved	GABA	
Mechanism of action	Potentiation of GABA interaction with $GABA_A$ receptors involves BZ_1 and BZ_2 binding sites	Prolongation of GABA, GABA mimetic at high doses, on $GABA_A$ receptors
Effects	Light to moderate CNS depression	Any plane of CNS depression

Table IV-8-1. Properties of Drugs of Abuse (continued)

CNS Depressants	Benzodiazepines	Barbiturates and Ethanol
Toxicity	Sedation, anterograde amnesia; in severe OD (or IV use), reverse with **flumazenil**	Severe CNS depression, respiratory depression, and death
Withdrawal	Rebound insomnia, rebound anxiety	Agitation, anxiety, hyperreflexia, and life-threatening seizures + in ethanol withdrawal delusions/ hallucinations—delirium tremens (DTs)
Opioids	**Morphine, Heroin, Methadone, Fentanyls, Other Opioids**	
Neurotransmitters involved	NE, DA, 5HT, GABA, and many others	
Mechanism of action	Activate opioid μ, κ, and δ receptors. Potent μ receptor activators have the most intense abuse and dependence liability, possibly effected via an increase in dopaminergic transmission in the mesolimbic tracts	
Effects	Euphoria, analgesia, sedation, cough suppression, and constipation; strong miosis (except meperidine)	
Toxicity	Severe respiratory depression (reverse with **naloxone**), nausea, vomiting	
Withdrawal	Lacrimation, yawning, sweating, and restlessness, rapidly followed with centrally originating pain, muscle cramping, and diarrhea; not life-threatening	
Hallucinogens	**Marijuana**	**Hallucinogens**
Neurotransmitters involved	Many	5HT
Mechanism of action	Interaction of THC with CB_1 and CB_2 cannabinoid receptors in CNS and periphery	Interaction with several subtypes of 5HT receptors
Effects	Sedation, euphoria, ↑ HR, conjunctival irritation, delusions, hallucinations	Hallucinogen, sympathomimetic, causes dysesthesias
Toxicity	Associated with smoking, possible flashbacks	Poorly described, flashbacks likely
Withdrawal	Irritability, anxiety	Poorly characterized
Miscellaneous Abused Drugs		
1. PCP: NMDA-receptor antagonist; extremely toxic, horizontal and vertical nystagmus, paranoia, rhabdomyolysis; overdose is common, with convulsions and death 2. Ketamine: similar to but milder than PCP, with hallucinations, glutamate-receptor antagonist 3. Anticholinergics: scopolamine, atropine-like 4. MDMA ("Ecstasy"), MDA, MDEA: amphetamine-like with strong 5HT pharmacology and therefore hallucinogenic; generally neurotoxic 5. Inhalants: solvent abuse, multiple organ damage; see Toxicology, section XI		

9 CNS Drug List and Practice Questions

Table IV-9-1. CNS Drug List

Sedative-Hypnotics	Anticonvulsants
Barbiturates: phenobarbital Benzodiazepines: alprazolam, diazepam, lorazepam, oxazepam	Carbamazepine, ethosuximide, valproic acid, phenytoin, diazepam, lorazepam, gabapentin, lamotrigine, felbamate, topiramate, tiagabine, vigabatrin
	Anesthetics (Inhaled)
Others: buspirone, zolpidem, zaleplon BZ receptor antagonist: flumazenil	Desflurane, sevoflurane, nitrous oxide
Anesthetics (IV)	**Neuromuscular Blocking Agents**
Fentanyl, ketamine, midazolam, propofol, thiopental	Depolarizing: succinylcholine Nondepolarizing: atracurium, tubocurarine
Local Anesthetics	**Skeletal Muscle Relaxants**
Lidocaine, bupivacaine, mepivacaine, procaine, cocaine	Depolarizing: succinylcholine Nondepolarizing: d-tubocurarine, atracurium
Opioid Analgesics	**Antipsychotics**
Full agonists: morphine, meperidine, methadone, fentanyl, and heroin Partial agonists: buprenorphine, codeine Mixed agonist-antagonists: nalbuphine Antagonists: naloxone, naltrexone, methylnaltrexone	Typicals: Chlorpromazine, fluphenazine, thioridazine, haloperidol Atypicals: clozapine, risperidone, olanzapine, aripiprazole, quetiapine, ziprasidone
Antiparkinsonian Drugs	**Antidepressants**
DA agonists: levodopa, bromocriptine, pramipexole MAO-B inhibitor: selegiline AAAD inhibitor: carbidopa M blockers: benztropine, trihexyphenidyl COMT inhibitor: tolcapone DA releaser and M blocker: amantadine	MAOIs: phenelzine, tranylcypromine TCAs: amitriptyline, imipramine, clomipramine SSRIs: fluoxetine, paroxetine, sertraline Others: bupropion, mirtazapine, trazodone, venlafaxine
Bipolar Disorder	**ADHD**
Lithium	Methylphenidate Atomoxetine

PRACTICE QUESTIONS

1. Lorazepam can be safely used as a preanesthetic medication in a patient undergoing liver transplantation without fear of excessive CNS depression because the drug is

   A. excreted in unchanged form
   B. actively secreted into the GI tract
   C. conjugated extrahepatically
   D. a selective anxiolytic devoid of CNS depressant actions
   E. reversible by naloxone

2. Midazolam is an effective anesthetic because it acts by

   A. increasing functional activity at $GABA_B$ receptors
   B. enhancing the actions of dopamine
   C. blocking the NMDA glutamate receptor subtype
   D. acting as a partial agonist at 5HT receptors
   E. facilitating GABA-mediated increases in chloride ion conductance

3. Which one of the following is an established clinical use of morphine?

   A. Management of generalized anxiety disorders
   B. Relief of pain associated with biliary colic
   C. Pulmonary congestion
   D. Treatment of cough associated with use of ACE inhibitors
   E. Suppression of the ethanol withdrawal syndrome

4. A 40-year-old man was given a drug that binds to a subunit of the $GABA_A$ receptor. When used at a high dose, the drug can open Cl^- channels independent of GABA. What drug was the man given?

   A. Diazepam
   B. Ethanol
   C. Phenobarbital
   D. Baclofen
   E. Dronabinol

5. Which one of the following is characteristic of both phenytoin and carbamazepine?

   A. Inhibition of hepatic cytochrome P450
   B. First-order elimination at high therapeutic doses
   C. Enhances the effects of oral contraceptives
   D. Safe to use in pregnancy
   E. Prevent sodium influx through fast sodium channels

6. A patient comes to the ER with a painful stab wound. The ER resident administers pentazocine for the pain. Soon after administration the patient experiences sweating, restlessness, and an increase in pain sensations. What is the most likely explanation for his symptoms?

   A. The patient is probably tolerant to pentazocine.
   B. The patient is a heroin addict.
   C. Pentazocine is an ineffective analgesic.
   D. Pentazocine was used at the wrong dose.
   E. Pentazocine doesn't cross the blood-brain barrier.

7. The data shown in the table below concern the effects of drugs on transmitter function in the CNS. Which one of the drugs is most likely to alleviate extrapyramidal dysfunction caused by typical antipsychotics? (The + signs denote intensity of drug actions.)

Drug	Activation of DA Receptors	Activation of GABA Receptors	Block of ACh M Receptors
A.	++++	0	0
B.	++	++	0
C.	0	0	++++
D.	0	+++++	0
E.	+	+	0

8. Tricyclic antidepressants

   A. have anticonvulsant activity
   B. should not be used in patients with glaucoma
   C. may increase oral absorption of levodopa
   D. are sometimes used as antiarrhythmics

9. Which one of the following statements about lithium is accurate?

   A. It causes symptoms of mild hyperthyroidism in up to 25% of patients.
   B. Plasma levels are increased by a high-Na diet.
   C. Adverse effects include acne, polydipsia, and polyuria.
   D. Spina bifida is major concern in fetal development.
   E. Sedative actions calm manic patients within 24 h.

10. Ingestion of methanol in wood spirits would cause which of the following to happen?

    A. The formation of formaldehyde
    B. Nephrotoxicity
    C. Hypotension and vomiting
    D. The production of glycolic acids
    E. Inhibition of aldehyde dehydrogenase

11. What Is the rationale for combining levodopa with carbidopa?

    A. Carbidopa stimulates dopamine receptors
    B. Carbidopa increases levodopa entry into the CNS by inhibiting peripheral dopa decarboxylase
    C. Carbidopa enhances levodopa absorption
    D. Carbidopa enhances the peripheral conversion of levodopa to dopamine
    E. Carbidopa blocks peripheral COMT

12. A 29-year-old man is being treated with an antidepressant drug, and his mood is improving. However, he complains of feeling "jittery" and agitated at times, and if he takes his medication in the afternoon he finds it difficult to get to sleep at night. He seems to have lost weight during the 6 months that he has been taking the drug. He has been warned not to take other drugs without consultation because severe reactions have occurred with opioid analgesics including meperidine. This patient is probably taking

    A. alprazolam
    B. chlorpromazine
    C. paroxetine
    D. amitriptyline
    E. trazodone

13. The ability of several drugs to inhibit the reuptake of CNS amine neurotransmitters is shown in the table below (number of arrows ↓ indicates the intensity of inhibitory actions). Which one of the drugs is most likely to have therapeutic effectiveness in the management of both obsessive-compulsive disorders (OCD) and major depressive disorders?

Drug	DA Reuptake	NE Reuptake	5HT Reuptake	GABA Reuptake
A.	↓↓	0	0	↓↓
B.	0	↓↓↓↓	↓	0
C.	0	0	↓↓↓↓	0
D.	0	0	↓	↓↓↓↓
E.	↓↓↓↓	↓↓	0	0

14. A patient suffering from attention deficit hyperactivity disorder is placed on atomoxetine. A drug that has a similar mechanism of action to atomoxetine is

A. methylphenidate
B. botulinum toxin
C. clonidine
D. amitriptyline
E. entacapone

15. A patient suffering from generalized anxiety disorder (GAD) has a history of drug dependence that includes the illicit use of secobarbital ("reds") and a variety of other drugs. Psychotherapy is indicated, but the physician also prescribes a drug that can be helpful in GAD and that has the advantage of no abuse liability. The drug prescribed was most likely to have been

A. bupropion
B. buspirone
C. baclofen
D. buprenorphine
E. phenobarbital

16. A patient has been diagnosed as having "long QT syndrome." The patient is experiencing significant pain following a bout with shingles. What would be an appropriate drug for his pain?

    A. Amitriptyline
    B. Fentanyl
    C. Acyclovir
    D. Diazepam
    E. Gabapentin

17. A habitual user of a schedule-controlled drug abruptly stops using it. Within 8 h, she becomes anxious, starts to sweat, and gets severe abdominal pain with diarrhea. These symptoms intensify over the next 12 h, during which time she has a runny nose, is lacrimating, and has uncontrollable yawning and intensification of muscle cramping and jerking. Assuming that these are withdrawal symptoms in the patient due to her physical dependence, the drug most likely to be involved is

    A. alprazolam
    B. amphetamine
    C. ethanol
    D. meperidine
    E. secobarbital

18. A 57-year-old patient, living at home, has severe pain due to a metastatic carcinoma that is being managed with fentanyl, delivered transdermally from a patch. He should also be taking, or at least have on hand

    A. apomorphine
    B. docusate
    C. loperamide
    D. morphine
    E. naloxone

19. A hospital nurse is taking imipramine for a phobic anxiety disorder, and her patient is being treated with chlorpromazine for a psychotic disorder. Which of the following adverse effects is likely to occur in both of these individuals?

    A. Excessive salivation
    B. Pupillary constriction
    C. Orthostatic hypotension
    D. Seizure threshold
    E. Weight loss

20. Which one of the following pairs of “drug/mechanism of action” is most accurate?

    A. Carbamazepine/facilitation of the actions of GABA
    B. Ethosuximide/blocks Na channels in axonal membranes
    C. Phenelzine/inhibits dopa decarboxylase
    D. Procaine/blocks Ca channels (type T) in thalamic neurons
    E. Lithium/inhibits recycling of inositol

21. A 30-year-old man is brought to the ER with the following symptoms attributed to a drug overdose: HR and BP, mydriasis, behavioral excitation, aggressiveness, paranoia, and hallucinations. Of the following drugs, which one is most likely to be responsible for these symptoms?

    A. Amphetamine
    B. Ethanol
    C. Fentanyl
    D. Flunitrazepam
    E. Marijuana

22. Which one of the following CNS receptors is directly coupled to an ion channel so that the effects of its activation do not involve second messenger systems?

    A. N(ACh)
    B. α (NE)
    C. D_{2A} (DA)
    D. μ (beta endorphin)
    E. $5HT_2$ (serotonin)

ANSWERS AND EXPLANATIONS

1. **Answer: C.** Most benzodiazepines are metabolized by liver cytochrome P450. In a patient lacking liver function, benzodiazepines that are metabolized via extrahepatic conjugation (e.g., lorazepam, oxazepam) are safer in terms of the possibility of excessive CNS depression. Lorazepam is metabolized, probably in the lungs, via glucuronidation. Although benzodiazepine actions can be reversed, the drug that acts as an antagonist is flumazenil, not naloxone.

2. **Answer: E.** Benzodiazepines interact with components of the GABA receptor–chloride ion channel macromolecular complex. Binding of BZs leads to an increase in the frequency of chloride ion channel opening elicited by the inhibitory transmitter GABA. Benzodiazepines do not act on $GABA_B$ receptors; baclofen, a centrally acting muscle relaxant, is an agonist at these receptors. Buspirone, the selective anxiolytic, may be a partial agonist at 5HT receptors.

3. **Answer: C.** Morphine continues to be used in pulmonary congestion, in part because of its sedative (calming) and analgesic effects and also because of its vasodilating actions, which result in favorable hemodynamics in terms of cardiac and pulmonary function. Similarly, morphine is of value in an acute MI, especially its ability to relieve pain. However, morphine is not suitable for pain of biliary origin because it causes contraction of the sphincters of Oddi, leading to spasms. None of the other proposed indications are appropriate.

4. **Answer: C.** Benzodiazepines, barbiturates, and ethanol all modulate the actions of the $GABA_A$ receptor, while baclofen works at the $GABA_B$ receptor, and dronabinol works on cannabinoid receptors. Of the $GABA_A$ drugs, only barbiturates have GABA-mimicking activity and this occurs at high doses. This is one of the reasons why barbiturates are a more dangerous group of drugs than benzodiazepines since benzos lack GABA-mimicking activity.

5. **Answer: E.** Phenytoin has the unusual characteristic of following first-order elimination kinetics at low doses but zero-order kinetics at high doses because of saturation of the liver enzymes involved in its metabolism. Carbamazepine, like most drugs, follows first-order kinetics. Both drugs are P450 inducers and can increase the metabolism of oral contraceptives making them less effective. Both drugs are teratogenic, causing structural abnormalities during fetal development including cleft palate. Both drugs block inactivated sodium channels, preventing sodium entry, thereby prolonging the time to recovery.

6. **Answer: B.** Pentazocine is an agonist at κ (kappa) opioid receptors and an <u>ant</u>agonist at μ opioid receptors. Mixed agonist-antagonists can displace μ receptor agonists such as heroin from receptors, resulting in the rapid development of symptoms of withdrawal in patients who are physically dependent on such drugs—"precipitated withdrawal." Symptoms include yawning, lacrimation, salivation, restlessness, anxiety, sweating, goosebumps, muscle cramps, and pain.

7. **Answer: C.** Muscarinic receptor antagonists such as benztropine, trihexyphenidyl, and diphenhydramine are used to manage the reversible extrapyramidal dysfunction (e.g., pseudo-Parkinsonism) that results from treatment with drugs that block DA receptors in the striatum (typical antipsychotics). Drugs that activate DA receptors, although theoretically possible, require doses that are toxic and exacerbate psychoses. Because the actions of DA in the striatum lead to inhibition of GABA-ergic neurons, drugs that activate GABA receptors are unlikely to be effective in this situation, although they may well have both anxiolytic and anticonvulsant properties.

8. **Answer: B.** In addition to blocking reuptake of NE and 5HT, pharmacodynamic actions of the tricyclic antidepressants include block of peripheral adrenergic and muscarinic receptors—the former resulting in postural hypotension and the latter, via mydriasis, exacerbating glaucoma. TCAs may cause arrhythmias in overdose. They have no effect on the absorption of levodopa.

9. **Answer: C.** Lithium causes goiter in a significant number of patients; however, thyroid dysfunction does not occur in all such patients, and when it does it presents as hypothyroidism (not hyper-T). High-Na diets increase lithium elimination; low Na increases lithium plasma levels. Uncoupling of vasopressin receptors is characteristic of lithium, leading to a nephrogenic diabetes insipidus. Although potential teratogenicity is a concern during pregnancy, lithium does not cause neural tube defects but may cause abnormalities in heart valves. Lithium takes 10 to 20 days for effectiveness, and in acute mania it is often necessary to calm the patient with parenteral antipsychotic drugs such as fluphenazine or haloperidol.

10. **Answer: A.** Methanol is metabolized by alcohol dehydrogenase to formaldehyde and then further metabolized to formic acid by aldehyde dehydrogenase. Its major toxicity is severe vision damage. Ethylene glycol Ingestion is associated with nephrotoxicity, while ethanol ingestion causes nausea, vomiting, and hypotension.

11. **Answer: B.** Carbidopa inhibits peripheral dopa decarboxylase which enhances uptake of levodopa into the CNS and therefore, its conversion to dopamine. Carbidopa doesn't cross the blood-brain barrier and therefore has no direct benefit at dopamine receptors.

12. **Answer: C.** The patient is probably taking an SSRI such as paroxetine. SSRIs rarely cause sedation and commonly cause agitation and the "jitters," which sometimes necessitates concomitant use of drugs that are strongly sedating, such as trazodone. SSRIs are best taken in the morning to avoid problems of insomnia, and they appear to cause weight loss, at least during the first 12 months of treatment. Severe drug interactions leading to the "serotonin syndrome" have been reported when SSRIs have been used together with MAO inhibitors, tricyclics, and the opioid meperidine.

13. **Answer: C.** Drug C appears to be a selective inhibitor of the reuptake of serotonin, and existing drugs of this class (SSRIs) are approved for use in both major depressive and obsessive-compulsive disorders. The tricyclic antidepressant clomipramine, a potent inhibitor of 5HT reuptake, was formerly the drug of choice for OCD until replaced by the SSRIs. Drugs A and E may have value in the treatment of Parkinson disease because they block the reuptake of DA. Drug D may be effective in anxiety and seizure states because it is an effective blocker of GABA reuptake.

14. **Answer: D.** Atomoxetine is used in attention deficit hyperactivity disorder (ADHD) and works by blocking the reuptake of norepinephrine into nerve terminals. This mechanism is how both cocaine and the tricyclic antidepressants such as amitriptyline work. Amphetamines such as methylphenidate are also commonly used in ADHD and work by displacing norepinephrine from the mobile pool.

15. **Answer: B.** Buspirone has selective anxiolytic activity that is slow in onset. The drug has no abuse liability and will not suppress withdrawal symptoms in patients who have become physically dependent on barbiturates, benzodiazepines, or ethanol. Bupropion is an antidepressant, also approved for management of dependence on nicotine. Baclofen is a spinal cord muscle relaxant that activates $GABA_B$ receptors. Buprenorphine is a long-acting opioid analgesic with no effectiveness in GAD, and phenobarbital is a barbiturate that may cause dependence.

16. **Answer: E.** The patient is experiencing postherpetic neuralgia. While acyclovir is effective at eradicating the herpes virus it is ineffective against the pain of shingles. Appropriate drugs are TCAs like amitriptyline and gabapentin. Patients with long QT syndrome have a genetic flaw in cardiac inward rectifying K current, leading to increased APD. Drugs that accentuate this by inhibiting the repolarizing K current (phase 3), which include thioridazine and the tricyclic antidepressants, are likely to have enhanced cardiotoxic potential in such patients. As a result, this patient should be placed on gabapentin.

17. **Answer: D.** The signs and symptoms described are typical of withdrawal from physical dependency on an opioid that has efficacy equivalent to a full agonist—in this case, meperidine. Although anxiety, agitation, and even muscle jerking may occur in withdrawal from dependence on sedative-hypnotics such as alprazolam and secobarbital, the symptoms of GI distress, rhinorrhea, lacrimation, and yawning are not characteristic (seizures are more typical). Symptoms of withdrawal from high-dose use of CNS stimulants such as amphetamine or cocaine include lassitude and severe depression of mood. The phrase “schedule-controlled” refers to FDA classifications of drugs that have abuse liability, including both licit and illicit drugs.

18. **Answer: B.** Fentanyl is a full agonist at opioid receptors and provides analgesia in cancer pain equivalent to morphine, so there is no good reason to have morphine on hand, and it would be a danger to the patient in terms of accidental overdose. Apomorphine is an emetic, hardly appropriate given the stimulatory effects of opioids on the emetic center. Likewise, loperamide is used in diarrheal states, and patients on strong opioids are almost certain to be constipated; for this reason, a stool softener like docusate should be available to the patient. The opioid antagonist naloxone is used IV in overdose situations but would not be provided to the patient for use PRN.

19. **Answer: C.** Orthostatic hypotension occurs with both tricyclic antidepressants and phenothiazines because both types of drug can block alpha-adrenergic receptors in venous beds. Their ability to block M receptors leads to xerostomia (not salivation) and mydriasis (not miosis). Tricyclics and phenothiazines also share a common tendency to decrease seizure threshold and cause weight gain (not loss).

20. **Answer: E.** Lithium inhibits the dephosphorylation of IP_2 (needed for the recycling of inositol), leading to depletion of membrane PIP_2. Consequently, the activation of receptors by neurotransmitters such as ACh, NE, and 5HT fails to release the second messengers IP_3 and DAG. Carbamazepine and the local anesthetic procaine block axonal Na channels; ethosuximide may block Ca channels in thalamic neurons. Phenelzine is a nonselective inhibitor of MAO.

21. **Answer: A.** The signs and symptoms are characteristic of a CNS stimulant that facilitates the activity of amines in both the CNS and the periphery. Amphetamines promote the release of NE from sympathetic nerve endings, causing CV stimulation and pupillary dilation. In the CNS, they enhance the actions of DA, NE, and 5HT, causing behavioral excitation and a psychotic state that may be difficult to distinguish from schizophrenia. Ethanol, marijuana, fentanyl, and flunitrazepam (a benzodiazepine that has been used in "date rape") are all CNS depressants.

22. **Answer: A.** ACh receptors in the CNS are present on less than 5% of the neuronal population. Most of them are of the muscarinic subtype, M_1 (excitatory) and M_2 (inhibitory), via G-protein coupled changes in cAMP. Nicotinic receptors are excitatory via direct coupling to cation channels (Na/K), and their activation does not initiate second messenger pathways. Other CNS transmitter receptors that are directly coupled to ion channels include those for GABA and glutamic acid. Almost all CNS receptors for DA, NE, 5HT, and opioid peptides are coupled to ion channels via second messenger systems.

PART V

Antimicrobial Agents

Antibacterial Agents 1

Learning Objectives

- ❑ Apply the principles of antimicrobial chemotherapy to select the best treatment
- ❑ Differentiate medications that inhibitor cell-wall synthesis, bacterial protein synthesis, and nucleic acid synthesis
- ❑ Answer questions about unclassified antibiotics
- ❑ Describe the differences between standard antibacterial agents and antitubercular drugs

PRINCIPLES OF ANTIMICROBIAL CHEMOTHERAPY

- Bactericidal
- Bacteriostatic
- Combinations: **additive**; **synergistic** (penicillins plus aminoglycosides); and **antagonistic** (penicillin plus tetracyclines)

Mechanisms

High-Yield

Table V-1-1. Mechanism of Action of Antimicrobial Agents

Mechanism of Action	Antimicrobial Agents
Inhibition of bacterial cell-wall synthesis	Penicillins, cephalosporins, imipenem/meropenem, aztreonam, vancomycin
Inhibition of bacterial protein synthesis	Aminoglycosides, chloramphenicol, macrolides, tetracyclines, streptogramins, linezolid
Inhibition of nucleic synthesis	Fluoroquinolones, rifampin
Inhibition of folic acid synthesis	Sulfonamides, trimethoprim, pyrimethamine

Resistance

Table V-1-2. Mechanisms of Resistance to Antimicrobial Agents

Antimicrobial Agents	Primary Mechanism(s) of Resistance
Penicillins and cephalosporins	Production of beta-lactamases, which cleave the beta-lactam ring structure; change in penicillin-binding proteins; change in porins
Aminoglycosides (gentamicin, streptomycin, amikacin, etc.)	Formation of enzymes that inactivate drugs via conjugation reactions that transfer acetyl, phosphoryl, or adenylyl groups
Macrolides (erythromycin, azithromycin, clarithromycin, etc.) and clindamycin	Formation of methyltransferases that alter drug binding sites on the 50S ribosomal subunit Active transport out of cells
Tetracyclines	Increased activity of transport systems that “pump” drugs out of the cell
Sulfonamides	Change in sensitivity to inhibition of target enzyme; increased formation of PABA; use of exogenous folic acid
Fluoroquinolones	Change in sensitivity to inhibition of target enzymes; increased activity of transport systems that promote drug efflux
Chloramphenicol	Formation of inactivating acetyltransferases

INHIBITORS OF CELL-WALL SYNTHESIS

All cell-wall synthesis inhibitors are bactericidal. They are divided into B-lactam and non-B-lactam drugs.

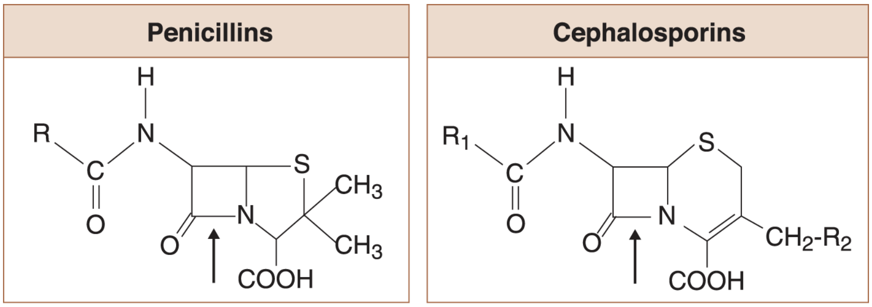


Figure V-1-1. Beta-Lactam Antibiotics

Penicillins

High-Yield

- Mechanisms of action:
  - Bacterial cell wall is cross-linked polymer of polysaccharides and pentapeptides
  - Penicillins interact with cytoplasmic membrane-binding proteins (PBPs) to inhibit transpeptidation reactions involved in cross-linking, the final steps in cell-wall synthesis
- Mechanisms of resistance:
  - Penicillinases (beta-lactamases) break lactam ring structure (e.g., staphylococci)
  - Structural change in PBPs (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA], penicillin-resistant pneumococci)
  - Change in porin structure (e.g., *Pseudomonas)*
- Subgroups and antimicrobial activity:
  - Narrow spectrum, beta-lactamase sensitive: penicillin G and penicillin V
    - Spectrum: streptococci, pneumococci, meningococci, *Treponema pallidum*
  - Very narrow spectrum, beta-lactamase resistant: nafcillin, methicillin, oxacillin
    - Spectrum: known or suspected staphylococci (not MRSA)
  - Broad spectrum, aminopenicillins, beta-lactamase sensitive: ampicillin and amoxicillin
    - Spectrum: gram-positive cocci (not staph), *E. coli*, *H. influenzae*, *Listeria monocytogenes* (ampicillin), *Borrelia burgdorferi* (amoxicillin), *H. pylori* (amoxicillin)
  - Extended spectrum, antipseudomonal, beta-lactamase sensitive: ticarcillin, piperacillin
    - Spectrum: increased activity against gram-negative rods, including *Pseudomonas aeruginosa*
- General considerations:
  - Activity enhanced if used in combination with beta-lactamase inhibitors (clavulanic acid, sulbactam)
  - Synergy with aminoglycosides against pseudomonal and enterococcal species
- Pharmacokinetics:
  - Most are eliminated via active tubular secretion with secretion blocked by probenecid; dose reduction needed only in major renal dysfunction
  - Nafcillin and oxacillin eliminated largely in bile; ampicillin undergoes enterohepatic cycling, but excreted by the kidney
  - Benzathine penicillin G—repository form (half-life of 2 weeks)

Bridge to Biochemistry

Suicide Inhibitors

Metabolism of a substrate by an enzyme to form a compound which irreversibly inhibits that enzyme. Penicillinase inhibitors, such as clavulanic acid and sulbactam, are **suicide inhibitors**.

Bridge to Immunology

Drug Hypersensitivity Reactions

I. IgE mediated: rapid onset; anaphylaxis, angioedema, laryngospasm

II. IgM and IgG antibodies fixed to cells: vasculitis, neutropenia, positive Coombs test

III. Immune complex formation: vasculitis, serum sickness, interstitial nephritis

IV. T-cell mediated: urticarial and maculopapular rashes, Stevens-Johnson syndrome

- Side effects:
  - Hypersensitivity
    - Incidence 5 to 7% with wide range of reactions (types I–IV). Urticarial skin rash common, but severe reactions, including anaphylaxis, are possible.
    - Assume complete cross-allergenicity between individual penicillins
  - Other:
    - GI distress (NVD), especially ampicillin
    - Jarisch-Herxheimer reaction in treatment of syphilis

Cephalosporins

High-Yield

- Mechanisms of action and resistance: identical to penicillins
- Subgroups and antimicrobial activity:
  - First generation: cefazolin, cephalexin
    - Spectrum: gram-positive cocci (not MRSA), *E. coli, Klebsiella pneumoniae*, and some *Proteus* species
    - Common use in surgical prophylaxis
    - Pharmacokinetics: none enter CNS
  - Second generation: cefotetan, cefaclor, cefuroxime
    - Spectrum: ↑ gram-negative coverage, including some anaerobes
    - Pharmacokinetics: no drugs enter the CNS, except cefuroxime
  - Third generation: ceftriaxone (IM) and cefotaxime (parenteral), cefdinir and cefixime (oral)
    - Spectrum: gram-positive and gram-negative cocci (*Neisseria gonorrhea*), plus many gram-negative rods
    - Pharmacokinetics: most enter CNS; important in empiric management of meningitis and sepsis
  - Fourth generation: cefepime (IV)
    - Even wider spectrum
    - Resistant to most beta-lactamases
    - Enters CNS
- Pharmacokinetics:
  - Renal clearance similar to penicillins, with active tubular secretion blocked by probenecid
  - Dose modification in renal dysfunction
  - Ceftriaxone is largely eliminated in the bile
- Side effects:
  - Hypersensitivity:
    - Incidence: 2%
    - Wide range, but rashes and drug fever most common

Clinical Correlate

Ceftaroline is an unclassified (fifth-generation) cephalosporin that can bind to the most often seen mutation of the PBP in MRSA.

Classic Clues

Organisms *not* covered by cephalosporins are “LAME”:

L*isteria monocytogenes*

Atypicals (e.g., *Chlamydia, Mycoplasma*)

MRSA

Enterococci

  - Positive Coombs test, but rarely hemolysis
  - Assume complete cross-allergenicity between individual cephalosporins and partial cross-allergenicity with penicillins (about 5%)
  - Most authorities recommend avoiding cephalosporins in patients allergic to penicillins (for gram-positive organisms, consider macrolides; for gram-negative rods, consider aztreonam)

Imipenem and Meropenem

- Mechanism of action:
  - Same as penicillins and cephalosporins
  - Resistant to beta-lactamases
- Spectrum:
  - Gram-positive cocci, gram-negative rods (e.g., *Enterobacter, Pseudomonas* spp.), and anaerobes
  - Important in-hospital agents for empiric use in severe life-threatening infections
- Pharmacokinetics:
  - Imipenem is given with cilastatin, a renal dehydropeptidase inhibitor, which inhibits imipenem's metabolism to a nephrotoxic metabolite
  - Both drugs undergo renal elimination— ↓ dose in renal dysfunction
- Side effects:
  - GI distress
  - Drug fever (partial cross-allergenicity with penicillins)
  - CNS effects, including seizures with imipenem in overdose or renal dysfunction

Aztreonam

- Mechanism of action:
  - Same as for penicillins and cephalosporins
  - Resistant to beta-lactamases
- Uses:
  - IV drug mainly active versus gram-negative rods
  - No cross-allergenicity with penicillins or cephalosporins

Recall Question

Which of the following changes will result in methicillin-resistant *S. aureus*?

A. Change in muramyl pentapeptide

B. Change in porin structure

C. Presence of penicillinase

D. Structural changes in penicillin-binding proteins (PBP)

Answer: D

Vancomycin

High-Yield

- Mechanism of action:
  - Binding at the D-ala-D-ala muramyl pentapeptide to sterically hinder the transglycosylation reactions (and indirectly preventing transpeptidation) involved in elongation of peptidoglycan chains
  - Does not interfere with PBPs
- Spectrum: MRSA, enterococci, *Clostridium difficile* (backup drug)
- Resistance:
  - Vancomycin-resistant staphylococcal (VRSA) and enterococcal (VRE) strains emerging
  - Enterococcal resistance involves change in the muramyl pentapeptide "target," such that the terminal D-ala is replaced by D-lactate
- Pharmacokinetics:
  - Used IV and orally (not absorbed) in colitis
  - Enters most tissues (e.g., bone), but not CNS
  - Eliminated by renal filtration (important to decrease dose in renal dysfunction)
- Side effects:
  - "Red man syndrome" (histamine release)
  - Ototoxicity (usually permanent, additive with other drugs)
  - Nephrotoxicity (mild, but additive with other drugs)

INHIBITORS OF BACTERIAL PROTEIN SYNTHESIS

Site of Action

High-Yield

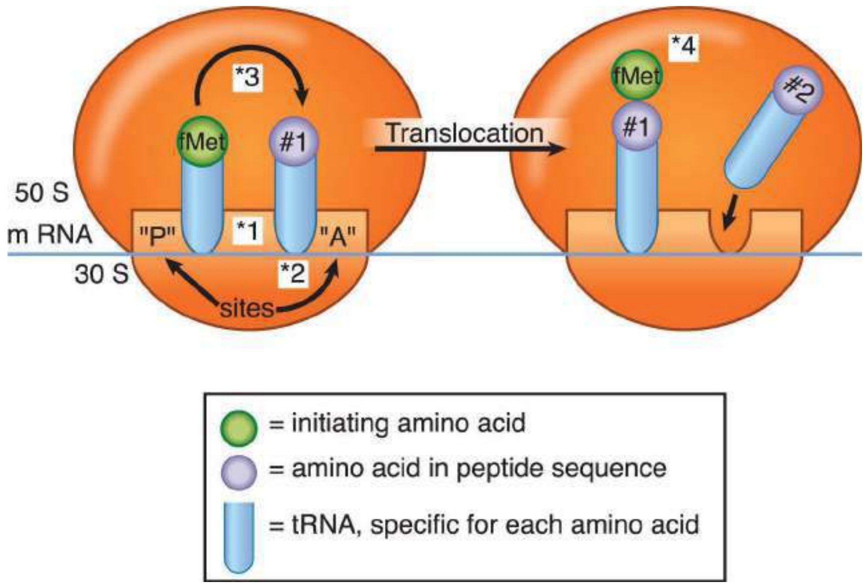


Figure V-1-2. Bacterial Protein Synthesis

Mechanisms

High-Yield

Table V-1-3. Mechanisms of Protein Synthesis Inhibition

Event	Antibiotic(s) and Binding Site(s)	Mechanism(s)
1. Formation of initiation complex	Aminoglycosides (30S) Linezolid (50S)	Interfere with initiation codon functions—block association of 50S ribosomal subunit with mRNA-30S (static); misreading of code (aminoglycosides only)—incorporation of wrong amino acid (–cidal)
2. Amino-acid incorporation	Tetracyclines (30S) Dalfopristin/ quinupristin (50S)	Block the attachment of aminoacyl tRNA to acceptor site (–static)
3. Formation of peptide bond	Chloramphenicol (50S)	Inhibit the activity of peptidyltransferase (–static)
4. Translocation	Macrolides and clindamycin (50S)	Inhibit translocation of peptidyl-tRNA from acceptor to donor site (–static)

- For mechanisms of resistance of antibiotics, see chapter 5.

Aminoglycosides

High-Yield

- Activity and clinical uses:
  - Bactericidal, accumulated intracellularly in microorganisms via an O_2-dependent uptake → anaerobes are innately resistant
  - Useful spectrum includes gram-negative rods; **gentamicin**, **tobramycin**, and **amikacin** often used in combinations
  - Synergistic actions occur for infections caused by enterococci (with penicillin G or ampicillin) and *P. aeruginosa* (with an extended-spectrum penicillin or third-generation cephalosporin)
  - **Streptomycin** used in tuberculosis; is the DOC for bubonic plague and tularemia
- Pharmacokinetics:
  - Are polar compounds, not absorbed orally or widely distributed into tissues
  - Renal elimination proportional to GFR, and major dose reduction needed in renal dysfunction
- Side effects:
  - Nephrotoxicity (6 to 7% incidence) includes proteinuria, hypokalemia, acidosis, and acute tubular necrosis—usually reversible, but enhanced by vancomycin, amphotericin B, cisplatin, and cyclosporine
  - Ototoxicity (2% incidence) from hair cell damage; includes deafness (irreversible) and vestibular dysfunction (reversible); toxicity may be enhanced by loop diuretics
  - Neuromuscular blockade with ↓ release of ACh—may enhance effects of skeletal muscle relaxants

Bridge to Microbiology

Once-Daily Dosing of Aminoglycosides

Antibacterial effects depend mainly on peak drug level (rather than time) and continue with blood levels < MIC—a postantibiotic effect (PAE).

Toxicity depends both on blood level and the time that such levels are > than a specific threshold (i.e., total dose).

Tetracyclines

High-Yield

- Activity and clinical uses:
  - Bacteriostatic drugs, actively taken up by susceptible bacteria
  - “Broad-spectrum” antibiotics, with good activity versus chlamydial and mycoplasmal species, *H. pylori* (GI ulcers), *Rickettsia, Borrelia burgdorferi, Brucella, Vibrio,* and *Treponema* (backup drug)
- Specific drugs:
  - **Doxycycline:** more activity overall than tetracycline HCl and has particular usefulness in prostatitis because it reaches high levels in prostatic fluid
  - **Minocycline:** in saliva and tears at high concentrations and used in the meningococcal carrier state
  - Tigecycline: used in complicated skin, soft tissue, and intestinal infections due to resistant gram + (MRSA, VREF), gram –, and anaerobes

Clinical Correlate

Don’t Use in Pregnancy

Aminoglycosides, fluoroquinolones, sulfonamides, tetracyclines

- Pharmacokinetics:
  - Kidney for most (↓ dose in renal dysfunction)
  - Liver for doxycycline
  - Chelators: tetracyclines bind divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}), which ↓ their absorption
- Side effects:
  - Tooth enamel dysplasia and possible ↓ bone growth in children (avoid)
  - Phototoxicity (demeclocycline, doxycycline)
  - GI distress (NVD), superinfections leading to candidiasis or colitis
  - Vestibular dysfunction (minocycline)
  - Have caused liver dysfunction during pregnancy at very high doses (contraindicated)

Chloramphenicol

- Activity and clinical uses:
  - Bacteriostatic with a wide spectrum of activity
  - Currently a backup drug for infections due to *Salmonella typhi, B. fragilis, Rickettsia*, and possibly in bacterial meningitis
- Pharmacokinetics:
  - Orally effective, with good tissue distribution, including CSF
  - Metabolized by hepatic glucuronidation, and dose reductions are needed in liver dysfunction and in neonates
  - Inhibition of cytochrome P450
- Side effects:
  - Dose-dependent bone marrow suppression common; aplastic anemia rare (1 in 35,000)
  - "Gray baby" syndrome in neonates (↓ glucuronosyl transferase)

Macrolides

High-Yield

- Drugs: erythromycin, azithromycin, clarithromycin
- Activity and clinical uses: wide-spectrum antibiotics
  - Gram-positive cocci (not MRSA)
  - Atypical organisms (*Chlamydia, Mycoplasma,* and *Ureaplasma* species)
  - *Legionella pneumophila*
  - *Campylobacter jejuni*
  - *Mycobacterium avium-intracellulare (MAC)*
  - *H. pylori*

Classic Clues

Phototoxicity

- Tetracyclines
- Sulfonamides
- Quinolones

Bridge to Microbiology

Community-Acquired Pneumonia

With no comorbidity, the most common organisms associated with community-acquired pneumonia are *M. pneumoniae*, *C. pneumoniae*, and viruses. In smokers, the pneumococcus is a more frequent pathogen. Macrolide antibiotics have activity against most strains of these organisms (other than viruses) and are therefore commonly used in the treatment of a community-acquired pneumonia.

- Pharmacokinetics: inhibit cytochrome P450s
- Side effects:
  - Gastrointestinal distress (erythromycin, azithromycin > clarithromycin) due to stimulation of motilin receptors
  - Reversible deafness at high doses
  - Increased QT interval
- Telithromycin: a ketolide active against macrolide-resistant *S. pneumonia*

Clindamycin

- Not a macrolide, but has the same mechanisms of action and resistance
- Narrow spectrum: gram-positive cocci (including community-acquired MRSA) and anaerobes, including *B. fragilis* (backup drug)
- Concentration in bone has clinical value in osteomyelitis due to gram-positive cocci
- Side effect: pseudomembranous colitis (most likely cause)

Linezolid

- Mechanism of action:
  - Inhibits the formation of the initiation complex in bacterial translation systems by preventing formation of the N-formylmethionyl-tRNA-ribosome-mRNA ternary complex
- Spectrum:
  - Treatment of VRSA, VRE, and drug-resistant pneumococci
- Side effects: bone marrow suppression (platelets), MAO-A and B inhibitor

Quinupristin–Dalfopristin

- Mechanism of action:
  - Quinupristin and dalfopristinstreptogramins that act in concert via several mechanisms
  - Binding to sites on 50S ribosomal subunit, they prevent the interaction of amino-acyl-tRNA with acceptor site and stimulate its dissociation from ternary complex
  - May also decrease the release of completed polypeptide by blocking its extrusion
- Spectrum:
  - Used parenterally in severe infections caused by vancomycin-resistant staphylococci (VRSA) and enterococci (VRE), as well as other drugresistant, gram-positive cocci
- Side effects:
  - Toxic potential remains to be established

Note

- Streptogramins for *E. faecium*, including VRE faecium, but not for *E. faecalis*
- Linezolid for both types of enterococci

INHIBITORS OF NUCLEIC ACID SYNTHESIS

Inhibitors of Folic Acid Synthesis

High-Yield

- Drugs: sulfonamides, trimethoprim, and pyrimethamine

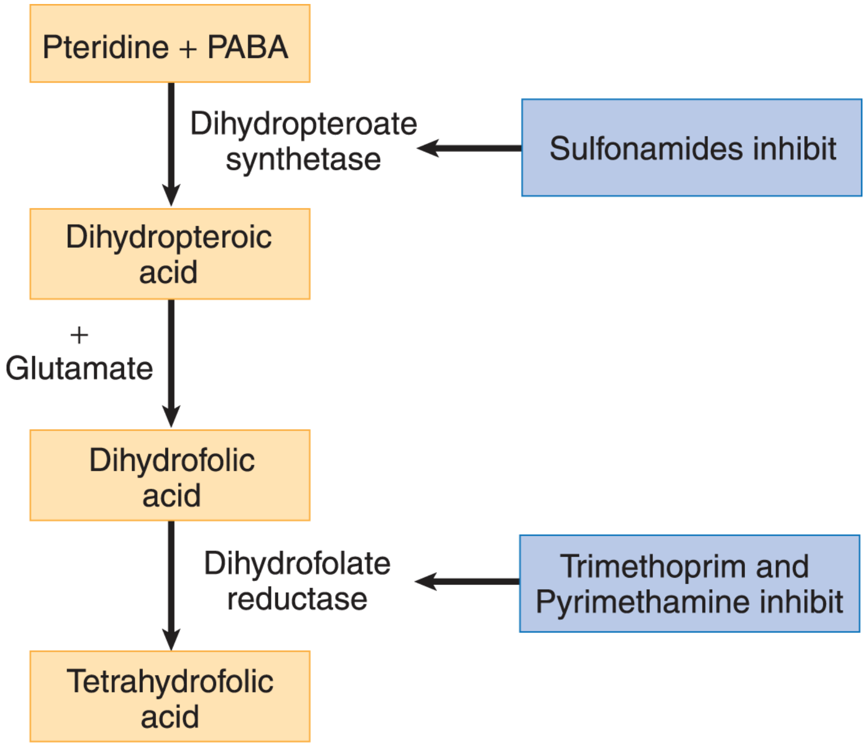


Figure V-1-3. Figure V-1-3. Inhibitors of Folic Acid Synthesis

- Activity and clinical uses:
  - Sulfonamides alone are limited in use because of multiple resistance
  - Sulfasalazine is a prodrug used in ulcerative colitis and rheumatoid arthritis (Figure V-1-4)
  - Ag sulfadiazine used in burns

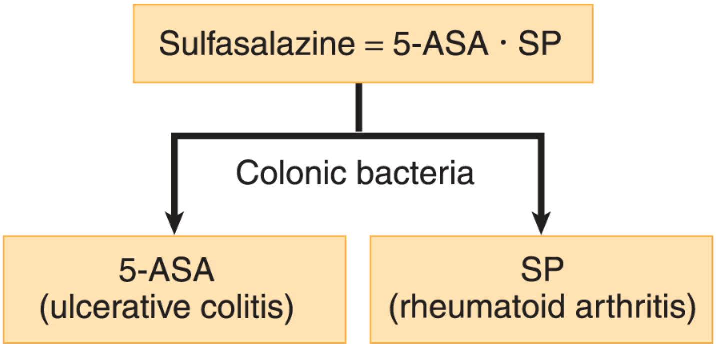


Figure V-1-4. Metabolism and Uses of Sulfasalazine

Bridge to Biochemistry

Antimetabolites are substances which inhibit cell growth by competing with, or substituting for, a natural substrate in an enzymatic process

Sulfonamides and trimethoprim are antimetabolites, as are many antiviral agents and drugs used in cancer chemotherapy.

5-ASA: 5-aminosalicylic acid

SP: sulfapyridine

- Combination with dihydrofolate reductase inhibitors:
  - ↓ resistance
  - Synergy
- Uses of trimethoprim-sulfamethoxazole (cotrimoxazole):
  - Bacteria:
    - DOC in *Nocardia*
    - *Listeria* (backup)
    - Gram-negative infections (*E. coli, Salmonella, Shigella, H. influenzae*)
    - Gram-positive infections (*Staph.*, including community-acquired MRSA, *Strep.*)
  - Fungus: *Pneumocystis jiroveci* (back-up drugs are pentamidine and atovaquone)
  - Protozoa: *Toxoplasma gondii* (sulfadiazine + pyrimethamine)

- Pharmacokinetics:
  - Sulfonamides are hepatically acetylated (conjugation)
  - Renally excreted metabolites cause crystalluria (older drugs)
  - High protein binding
    - Drug interaction
    - Kernicterus in neonates (avoid in third trimester)
- Side effects:
  - Sulfonamides
    - Hypersensitivity (rashes, Stevens-Johnson syndrome)
    - Hemolysis in G6PD deficiency
    - Phototoxicity
  - Trimethoprim or pyrimethamine
    - Bone marrow suppression (leukopenia)

Direct Inhibitors of Nucleic Acid Synthesis: Quinolones

- Drugs: ciprofloxacin, levofloxacin, and other "–floxacins"
- Mechanisms of action:
  - Quinolones are bactericidal and interfere with DNA synthesis
  - Inhibit topoisomerase II (DNA gyrase) and topoisomerase IV (responsible for separation of replicated DNA during cell division)
  - Resistance is increasing
- Activity and clinical uses:
  - Urinary tract infections (UTIs), particularly when resistant to cotrimoxazole
  - Sexually transmitted diseases (STDs)/pelvic inflammatory diseases (PIDs): chlamydia, gonorrhea

Note

The activity of quinolones includes *Bacillus anthracis*. Anthrax can also be treated with penicillins or tetracyclines.

- Skin, soft tissue, and bone infections by gram-negative organisms
- Diarrhea to *Shigella*, *Salmonella*, *E. coli*, *Campylobacter*
- Drug-resistant pneumococci (levofloxacin)

- Pharmacokinetics:
  - Iron, calcium limit their absorption
  - Eliminated mainly by kidney by filtration and active secretion (inhibited by probenecid)
  - Reduce dose in renal dysfunction
- Side effects:
  - Tendonitis, tendon rupture
  - Phototoxicity, rashes
  - CNS effects (insomnia, dizziness, headache)
  - Contraindicated in pregnancy and in children (inhibition of chondrogenesis)

UNCLASSIFIED ANTIBIOTIC

Metronidazole

High-Yield

- In anaerobes, converted to free radicals by ferredoxin, binds to DNA and other macromolecules, bactericidal
- Antiprotozoal: *Giardia*, *Trichomonas*, *Entamoeba*
- Antibacterial: strong activity against most anaerobic gram-negative *Bacteroides* species *Clostridium* species (DOC in pseudomembranous colitis), *Gardnerella*, and *H. pylori*
- Side effects: metallic taste, disulfiram-like effect

Clinical Correlate

Antibiotics for *H. pylori* Gastrointestinal Ulcers

- "BMT" regimen: bismuth, metronidazole, and tetracycline
- Clarithromycin, amoxicillin, omeprazole

ANTITUBERCULAR DRUGS

Combination drug therapy is the rule to delay or prevent the emergence of resistance and to provide additive (possibly synergistic) effects against *Mycobacterium tuberculosis*.

- The primary drugs in combination regimens are isoniazid (INH), rifampin, ethambutol, and pyrazinamide. Regimens may include 2–4 of these drugs, but in the case of highly resistant organisms, other agents may also be required. Backup drugs include aminoglycosides (streptomycin, amikacin, kanamycin), fluoroquinolones, capreomycin (marked hearing loss), and cycloserine (neurotoxic).
- Prophylaxis: usually INH, but rifampin if intolerant. In suspected multidrug resistance, both drugs may be used in combination.

Features of Antitubercular Drugs

High-Yield

Table V-1-4. Actions, Resistance, and Side Effects of Antitubercular Drugs

Drug	Mechanisms of Action and Resistance	Side Effects
Isoniazid (INH)	• Inhibits mycolic acid synthesis • Prodrug requiring conversion by catalase • High level resistance—deletions in *katG* gene (encodes catalase needed for INH bioactivation)	• Hepatitis (age-dependent) • Peripheral neuritis (use vitamin B6) • Sideroblastic anemia (use vitamin B6) • SLE in slow acetylators (rare)
Rifampin	• Inhibits DNA-dependent RNA polymerase (nucleic acid synthesis inhibitor)	• Hepatitis • Induction of P450 • Red-orange metabolites
Ethambutol	• Inhibits synthesis of arabinogalactan (cell-wall component)	• Dose-dependent retrobulbar neuritis → ↓ visual acuity and red-green discrimination
Pyrazinamide		• Hepatitis • Hyperuricemia
Streptomycin	• Protein synthesis inhibition (*see* Aminoglycosides)	• Deafness • Vestibular dysfunction • Nephrotoxicity

Recall Question

Which of the following is a side effect of linezolid?

A. Bone marrow suppression

B. Cardiotoxicity

C. Nephrotoxicity

D. Retinopathy

Answer: A

Antifungal Agents 2

Learning Objectives

❑ Demonstrate understanding of the use and side effects of polyenes (amphotericin B, nystatin), azoles (ketoconazole, fluconazole, itraconazole, voriconazole), and other antifungals

PROPERTIES OF ANTIFUNGAL DRUGS

Mechanism of Action

High-Yield

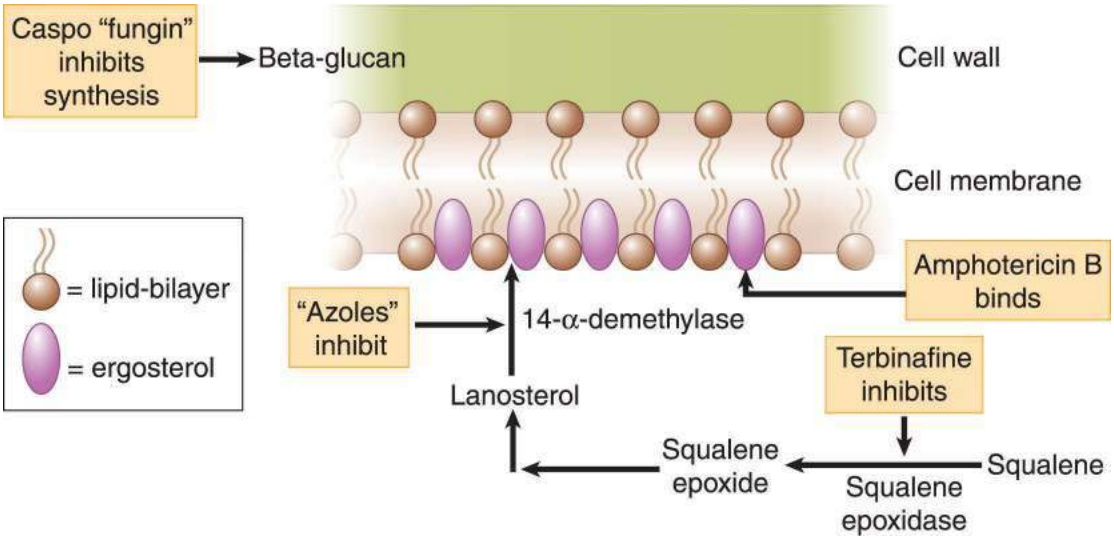


Figure V-2-1. Mechanism of Action of Antifungal Drugs

Polyenes (Amphotericin B, Nystatin)

- Mechanisms:
  - Amphoteric compounds with both polar and nonpolar structural components: interact with **ergosterol** in fungal membranes to form artificial "pores," which disrupt membrane permeability
  - Resistant fungal strains appear to have low ergosterol content in their cell membranes

- Activity and clinical uses:
  - Amphotericin B (Amp B) has wide fungicidal spectrum; remains the DOC (or co-DOC) for severe infection caused by *Cryptococcus* and *Mucor* (is synergistic with flucytosine in cryptococcoses)
  - Nystatin (too toxic for systemic use): used topically for localized infections (e.g., candidiasis)
- Pharmacokinetics:
  - Amp B given by slow IV infusion: poor penetration into the CNS (intrathecal possible)
  - Slow clearance (half-life >2 weeks) via both metabolism and renal elimination
- Side effects:
  - Infusion-related
    - Fever, chills, muscle rigor, hypotension (histamine release) occur during IV infusion (a test dose is advisable)
    - Can be alleviated partly by pretreatment with NSAIDs, antihistamines, meperidine, and adrenal steroids
- Dose-dependent
  - Nephrotoxicity includes ↓ GFR, tubular acidosis, ↓ K^+ and Mg^{2+}, and anemia through ↓ erythropoietin
  - Protect by Na^+ loading, use of liposomal amp B, or by drug combinations (e.g., + flucytosine), permitting ↓ in amp B dose

Azoles (Ketoconazole, Fluconazole, Itraconazole, Voriconazole)

- Mechanism:
  - "Azoles" are fungicidal and interfere with the synthesis of ergosterol by inhibiting 14-α-demethylase, a fungal P450 enzyme, which converts lanosterol to ergosterol
  - Resistance occurs via decreased intracellular accumulation of azoles
- Activity and clinical uses:
  - Ketoconazole
    - Co-DOC for *Paracoccidioides* and backup for *Blastomyces* and *Histoplasma*
    - Oral use in mucocutaneous candidiasis or dermatophytoses
  - Fluconazole
    - DOC for esophageal and invasive candidiasis and coccidioidomycoses
    - Prophylaxis and suppression in cryptococcal meningitis
  - Itraconazole and Voriconazole
    - DOC in blastomycoses, sporotrichoses, aspergillosis
    - Backup for several other mycoses and candidiasis

- Clotrimazole and miconazole
    - Used topically for candidal and dermatophytic infections
- Pharmacokinetics:
    - Effective orally
    - Absorption of ketoconazole ↓ by antacids
    - Absorption of itraconazole ↑ by food
    - Only fluconazole penetrates into the CSF and can be used in meningeal infection; fluconazole is eliminated in the urine, largely in unchanged form
    - Ketoconazole and itraconazole are metabolized by liver enzymes.
    - Inhibition of hepatic P450s
- Side effects: decreased synthesis of steroids, including cortisol and testosterone →↓ libido, gynecomastia, menstrual irregularities; decreased liver function tests and rare hepatotoxicity

Other Antifungals

- Flucytosine
    - Activated by fungal cytosine deaminase to 5-fluorouracil (5-FU), which after triphosphorylation is incorporated into fungal RNA
    - 5-FU also forms 5-fluorodeoxyuridine monophosphate (5-Fd-UMP), which inhibits thymidylate synthase →↓ thymine.
    - Resistance emerges rapidly if flucytosine is used alone.
    - Use in combination with amphotericin B in severe candidal and cryptococcal infections—enters CSF
    - Toxic to bone marrow (*see* Anticancer Drugs, Section IX).
- Griseofulvin
    - Active only against dermatophytes (orally, not topically) by depositing in newly formed keratin and disrupting microtubule structure
    - Side effects: disulfiram-like reaction
- Terbinafine
    - Active only against dermatophytes by inhibiting squalene epoxidase →↓ ergosterol
    - Possibly superior to griseofulvin in onychomycoses
    - Side effects: GI distress, rash, headache, ↑ liver function tests → possible hepatotoxicity
- Echinocandins (caspofungin and other "fungins")
    - Inhibit the synthesis of beta-1,2 glucan, a critical component of - fungal cell walls
    - Back-up drugs given IV for disseminated and mucocutaneous *Candida* infections or invasive aspergillosis
    - Monitor liver function

Antiviral Agents 3

Learning Objectives

- ❑ Answer questions about anti-herpetics and other antiviral agents
- ❑ Describe the appropriate treatment of HIV
- ❑ Solve problems concerning fusion inhibitors

ANTIVIRAL DRUG PROPERTIES

Many antiviral drugs are antimetabolites which resemble the structure of naturally occurring purine and pyrimidine bases or their nucleoside forms. Antimetabolites are usually prodrugs requiring metabolic activation by host-cell or viral enzymes; commonly, such bioactivation involves phosphorylation reactions catalyzed by kinases.

Sites of Action

High-Yield

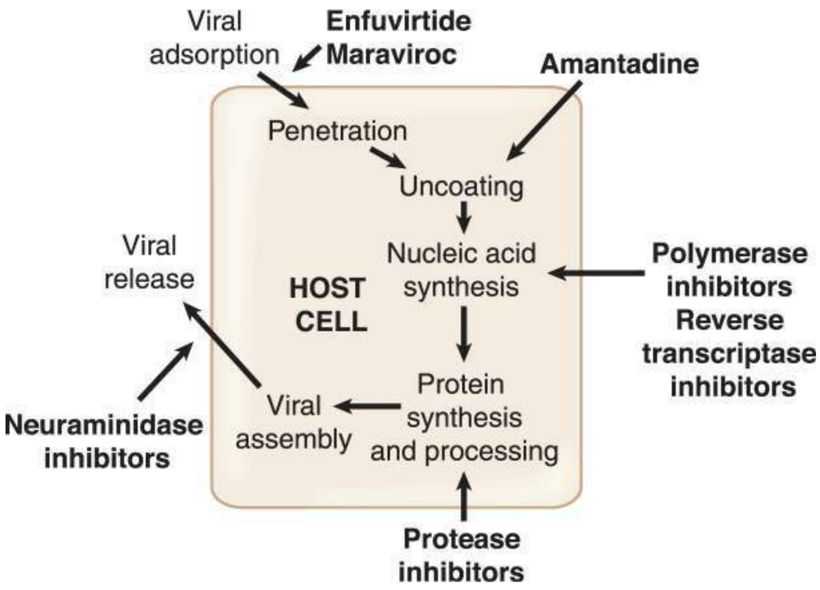


Figure V-3-1. Sites of Antiviral Drug Actions

Table V-3-1. Mechanism of Action of Antiviral Drugs

Mechanism of Action	Major Drugs
Block viral penetration/uncoating	Amantadine, enfuvirtide, maraviroc
Inhibit viral DNA polymerases	Acyclovir, foscarnet, ganciclovir
Inhibit viral RNA polymerases	Foscarnet, ribavirin
Inhibit viral reverse transcriptase	Zidovudine, didanosine, zalcitabine, lamivudine, stavudine, nevirapine, efavirenz
Inhibit viral aspartate protease	Indinavir, ritonavir, saquinavir, nelfinavir
Inhibit viral neuraminidase	Zanamivir, oseltamivir

ANTIHERPETICS

Acyclovir

High-Yield

- Mechanisms of action:
  - Monophosphorylated by viral thymidine kinase (TK), then further bioactivated by host-cell kinases to the triphosphate
  - Acyclovir-triphosphate is both a substrate for and inhibitor of viral DNA polymerase
  - When incorporated into the DNA molecule, acts as a chain terminator because it lacks the equivalent of a ribosyl 3′ hydroxyl group
  - Resistance possibly due to changes in DNA polymerase or to decreased activity of TK
  - >50% of HSV strains resistant to acyclovir completely lack thymidine kinase (TK^- strains)

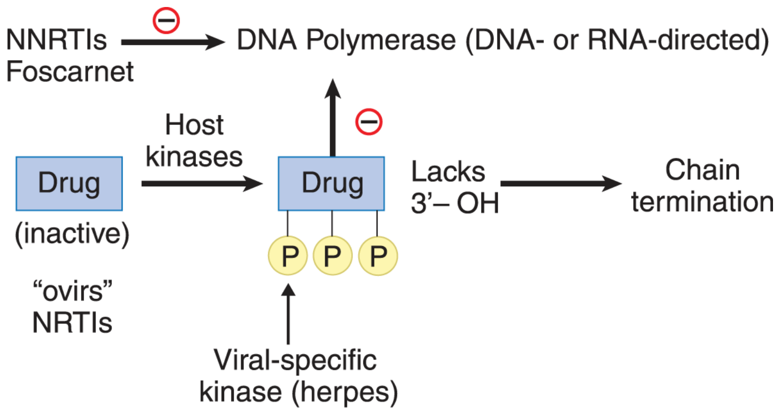


Figure V-3-2. Common Mechanism for "ovirs" and NRTIs

- Activity and clinical uses:
  - Activity includes herpes simplex virus (HSV) and varicella-zoster virus (VZV)
  - There are topical, oral, and IV forms; has a short half-life
  - Reduces viral shedding in genital herpes; ↓ acute neuritis in shingles but has no effect on postherpetic neuralgia
  - Reduces symptoms if used early in chickenpox; prophylactic in immunocompromised patients
- Side effects: minor with oral use, more obvious with IV; crystalluria (maintain full hydration) and neurotoxicity (agitation, headache, confusion [seizures in OD]); is **not** hematotoxic
- Newer drugs: famciclovir and valacyclovir are approved for HSV infection and are similar to acyclovir in mechanism (may have activity against strains resistant to acyclovir but not TK^- strains; may have longer $t_{1/2}$ than acyclovir)

Ganciclovir

- Mechanisms of action:
  - Similar to that of acyclovir
  - First phosphorylation step is viral-specific; involves thymidine kinase in HSV and a phosphotransferase (UL97) in cytomegalovirus (CMV)
  - Triphosphate form inhibits viral DNA polymerase and causes chain termination
  - Resistance mechanisms similar to acyclovir
- Activity and clinical uses:
  - HSV, VZV, and CMV
  - Mostly used in prophylaxis and treatment of CMV infections, including retinitis, in AIDS and transplant patients—relapses and retinal detachment occur
- Side effects: dose-limiting hematotoxicity (leukopenia, thrombocytopenia), mucositis, fever, rash, crystalluria (maintain hydration); seizures in overdose

Foscarnet

- Mechanisms and clinical uses:
  - Not an antimetabolite, but still inhibits viral DNA and RNA polymerases
  - Uses identical to ganciclovir, plus > activity versus acyclovir-resistant strains of HSV
- Side effects: dose-limiting nephrotoxicity with acute tubular necrosis, electrolyte imbalance with hypocalcemia (tremors and seizures); avoid pentamidine IV (→↑ nephrotoxicity and hypocalcemia)

TREATMENT OF HIV

Reverse Transcriptase Inhibitors (RTIs)

The original inhibitors of reverse transcriptases of HIV are nucleoside antimetabolites (e.g., zidovudine, the prototype) that are converted to active forms via phosphorylation reactions.

- **Nucleoside reverse transcriptase inhibitors (NRTIs):**
  - Are components of most combination drug regimens used in HIV infection
  - Are used together with a protease inhibitor (PI)
  - Highly active antiretroviral therapy (HAART) has often resulted in ↓ viral RNA, reversal of the decline in CD4 cells, and ↓ opportunistic infections
- **Nonnucleoside reverse transcriptase inhibitors (NNRTIs):**
  - RTIs that do not require metabolic activation: nevirapine, efavirenz
  - Are not myelosuppressant
  - Inhibit reverse transcriptase at a site different from the one NRTIs bind to
  - Additive or synergistic if used in combination with NRTIs and/or PIs

Zidovudine (Azidothymidine, ZDV, AZT)

- Mechanisms of action:
  - Phosphorylated nonspecifically to a triphosphate that can inhibit reverse transcriptase (RT) by competing with natural nucleotides and can also be incorporated into viral DNA to cause chain termination.
  - Resistance occurs by mutations (multiple) in the gene that codes for RT.

Other NRTIs

- Mechanism of action identical to that of zidovudine
- Each requires metabolic activation to nucleotide forms that inhibit reverse transcriptase
- Resistance mechanisms are similar
- Not complete cross-resistance between NRTIs
- Drugs differ in their toxicity profiles and are less bone-marrow suppressing than AZT
- Side effects:

Clinical Correlate

Tenofovir is an NtRTI commonly coformulated with an NRTI. Tenofovir has a single phosphate on its sugar residue and must be further phosphorylated to the triphosphate form.

Table V-3-2. Side Effects of NRTIs

Drug	Side Effects
Zidovudine, AZT	• Hematotoxicity (major and dose-limiting) • Headache, asthenia, myalgia, myopathy, and peripheral neuropathy
Didanosine, DDI	• Pancreatitis (major and dose-limiting) • Peripheral neuropathy, hyperuricemia, liver dysfunction
Lamivudine, 3TC; emtricitabine, FTC	• Least toxic of the NRTIs, but some GI effects and neutropenia • Active in hepatitis B (lamivudine)

Protease Inhibitors (PI)

- Mechanisms of action:
  - Aspartate protease (*pol* gene encoded) is a viral enzyme that cleaves precursor polypeptides in HIV buds to form the proteins of the mature virus core.
  - The enzyme contains a dipeptide structure not seen in mammalian proteins. PIs bind to this dipeptide, inhibiting the enzyme.
  - Resistance occurs via specific point mutations in the *pol* gene, such that there is not complete cross-resistance between different PIs.
- Clinical uses:
  - Ritonavir and other *-avirs*
  - Lopinavir, atazanavir, and darunavir are the PIs most commonly co-formulated with ritonavir
- Side effects:
  - Indinavir
    - Crystalluria (maintain hydration)
  - Ritonavir
    - Major drug interactions: induces CYP 1A2 and inhibits the major P450 isoforms (3A4 and 2D6)
  - General: syndrome of disordered lipid and CHO metabolism with central adiposity and insulin resistance

Integrase Inhibitors

- Mechanism of action: prevents integration of viral genome in host cell DNA
  - Raltegravir

Clinical Correlate

HIV Prophylaxis

Postexposure prophylaxis: emtricitabine + tenofovir + raltegravir

Pregnancy: 2 NRTIs (emtricitabine or lamivudine) + (zidovudine or tenofovir) + ritonavir-boosted atazanavir or lopinavir

Fusion Inhibitors

- Enfuvirtide: binds to gp41 and inhibits the fusion HIV-1 to CD4+ cells
- Maraviroc: blocks the binding of the gp120 HIV protein to CCR5 on macrophage surface to prevent viral entry
- Enfuvirtide and maraviroc block the entry of HIV into cells.

Note

Amantadine and rimantadine are no longer recommended as prophylaxis or treatment for influenza A viruses.

OTHER ANTIVIRALS

Zanamivir and Oseltamivir

- Mechanisms of action:
  - Inhibit neuraminidases of influenza A and B (enzymes that prevent clumping of virions so that more particles are available for infecting host cells)
  - Decreases likelihood that the virus will penetrate uninfected cells
- Clinical uses: prophylaxis mainly, but may decrease duration of flu symptoms by 2–3 days

Ribavirin

- Mechanisms:
  - Monophosphorylated form inhibits IMP dehydrogenase
  - Triphosphate inhibits viral RNA polymerase and end-capping of viral RNA
- Clinical uses:
  - Adjunct to alpha-interferons in hepatitis C
  - Management of respiratory syncytial virus
  - Lassa fever
  - Hantavirus
- Side effects:
  - Hematotoxic
  - Upper airway irritation
  - Teratogenic

Hepatitis C Treatment

- Sofosbuvir: nucleotide analog that inhibits RNA polymerase; combined with ribavirin or INT-α
- Simeprevir: hepatitis C protease inhibitor; combined with ribavirin or INT-α
- Ledipasvir: inhibits HCV NS5A protein that plays a key role in RNA replication; combined with sofosbuvir without INT-α or ribavirin

Recall Question

Which of the following best describes the mechanism of action of ribavirin?

A. Inhibits viral aspartate protease

B. Inhibits viral RNA polymerases

C. Inhibits viral DNA polymerases

D. Inhibits viral reverse transcriptase

Answer: B

Antiprotozoal Agents 4

Learning Objectives

❑ Demonstrate understanding of drugs for malaria and helminthic infections

OVERVIEW

Table V-4-1. Major Protozoal Infections and the Drugs of Choice

Infection	Drug of Choice	Comments
Amebiasis	Metronidazole	Diloxanide for noninvasive intestinal amebiasis
Giardiasis	Metronidazole	"Backpacker's diarrhea" from contaminated water or food
Trichomoniasis	Metronidazole	Treat both partners
Toxoplasmosis	Pyrimethamine + sulfadiazine	—
Leishmaniasis	Stibogluconate	—
Trypanosomiasis	Nifurtimox (Chagas disease) Arsenicals (African)	—

Antimalarial Drugs

- Clinical uses:
  - Chloroquine-sensitive regions
    - Prophylaxis: chloroquine +/– primaquine
    - Backup drugs: hydroxychloroquine, primaquine, pyrimethamine-sulfadoxine

- Specific treatment:

Table V-4-2. Treatment of Chloroquine-Sensitive Malaria

P. falciparum	Chloroquine
P. malariae	Chloroquine
P. vivax	Chloroquine + primaquine
P. ovale	Chloroquine + primaquine

  - Chloroquine-resistant regions
    - Prophylaxis: mefloquine; backup drugs: doxycycline, atovaquone-proguanil
    - Treatment: quinine +/– either doxycycline or clindamycin or pyrimethamine
- Side effects:
  - Hemolytic anemia in G6PD deficiency (primaquine, quinine)
  - Cinchonism (quinine)

Drugs for Helminthic Infections

- Most intestinal nematodes (worms)
  - Albendazole (↓ glucose uptake and ↓ microtubular structure)
  - Pyrantel pamoate (N_M agonist → spastic paralysis)
- Most cestodes (tapeworms) and trematodes (flukes)
  - Praziquantel (↑ Ca^{2+} influx, ↑ vacuolization)

Antimicrobial Drug List and Practice Questions

5

Table V-5-1. Antimicrobial Drug List

Penicillins	Cephalosporins	Other Cell Wall Inhibitors	
Penicillin G Nafcillin, oxacillin Amoxicillin, ampicillin Ticarcillin, piperacillin	Cefazolin (1st) Cefaclor (2nd) Ceftriaxone (3rd)	Imipenem, meropenem Vancomycin	
Macrolides	**Aminoglycosides**	**Tetracyclines**	**Others**
Erythromycin Azithromycin Clarithromycin	Gentamicin Tobramycin Streptomycin	Tetracycline HCl Doxycycline	Metronidazole
Fluoroquinolones	**Antifolates**	**Antimycobacterials**	
Ciprofloxacin Levofloxacin	Sulfamethoxazole Trimethoprim	Isoniazid, rifampin Ethambutol, pyrazinamide	
Antifungals	**Anti-Herpes**	**Anti-HIV**	
Amphotericin B Ketoconazole Fluconazole	Acyclovir Ganciclovir Foscarnet	Zidovudine (NRTI), didanosine (NRTI) Lamivudine (NRTI) Indinavir (PI), ritonavir (PI) Enfuvirtide, maraviroc	

PRACTICE QUESTIONS

1. A patient suffering from invasive aspergillosis is first administered NSAIDs, antihistamines, and adrenal glucocorticoids prior to administration of an antifungal drug. The antifungal drug works by

   A. binding to tubulin
   B. inhibiting squalene epoxidase
   C. inhibiting thymine synthesis
   D. binding to ergosterol
   E. inhibiting 14α-demethylase

2. A patient is prescribed isoniazid prophylactically since another family member currently has tuberculosis. When the patient ends up getting tuberculosis despite prophylaxis, resistance to isoniazid is suspected. In what way did this resistance likely develop?

   A. Decreased intracellular accumulation of the drug
   B. Inactivation of the drug via *N*-acetyltransferases
   C. Increased synthesis of mycolic acids
   D. Mutations in the gene coding for DNA-dependent RNA polymerase
   E. Reduced expression of the gene that encodes a catalase

3. A 7-year-old child presents with pharyngitis and fever of 2 days' duration, and microbiology reveals small, translucent, beta-hemolytic colonies sensitive in vitro to bacitracin. Past history includes a severe allergic reaction to amoxicillin when used for an ear infection. The physician needs to treat this infection but prefers not to use a drug that needs parenteral administration. Which one of the following agents is most likely to be appropriate in terms of both effectiveness and safety?

   A. Azithromycin
   B. Cefaclor
   C. Doxycycline
   D. Penicillin G
   E. Vancomycin

4. A woman has a sexually transmitted disease, and the decision is made to treat her with antibiotics as an outpatient. She is warned that unpleasant reactions may occur if she consumes alcoholic beverages while taking this drug. The antibiotic can be identified as which of the following?

   A. Ceftriaxone
   B. Doxycycline
   C. Metronidazole
   D. Ofloxacin
   E. Pen G

5. An 82-year-old hospitalized patient with creatinine clearance of 25 mL/min has a microbial infection requiring treatment with antibiotics. Which of the following drugs is least likely to require a dosage adjustment, either a smaller dose than usual or an increased interval between doses?

   A. Amphotericin B
   B. Ceftriaxone
   C. Gentamicin
   D. Imipenem-cilastatin
   E. Vancomycin

6. What drug is most likely to be effective in most diseases caused by nematodes?

   A. Chloroquine
   B. Mebendazole
   C. Metronidazole
   D. Praziquantel
   E. Pyrimethamine

7. What antibiotic effectively treats a variety of causative organisms for bacterial pneumonia, and also works at the 50S ribosomal subunit?

   A. Azithromycin
   B. Ceftriaxone
   C. Doxycycline
   D. Ofloxacin
   E. Clindamycin

8. In bacterial meningitis, third-generation cephalosporins are common drugs of choice. However, in neonatal meningitis they would not provide coverage if the infection was due to which of the following organisms?

   A. Meningococci
   B. *L. monocytogenes*
   C. Pneumococci
   D. *E. coli*
   E. Group B streptococci

9. Which one of the following drugs inhibits bacterial protein synthesis, preventing the translocation step via its interaction with the 50S ribosomal subunit?

   A. Clindamycin
   B. Gentamicin
   C. Chloramphenicol
   D. Imipenem
   E. Tetracycline

10. Which of the following is a mechanism underlying the resistance of strains of *S. pneumoniae* to the widely used antibiotic ciprofloxacin?

    A. Reduced topoisomerase sensitivity to inhibitors
    B. Increased synthesis of PABA
    C. Formation of methyltransferases that change receptor structure
    D. Structural changes in porins
    E. Formation of drug-inactivating hydrolases

11. Gentamicin would be an ineffective drug for which of the following organisms?

    A. *E. coli*
    B. *B. fragilis*
    C. *Pseudomonas*
    D. *Listeria* if combined with ampicillin
    E. *Proteus*

12. In the treatment of a urinary tract infection in a patient known to have a deficiency of glucose-6-phosphate dehydrogenase, it would not be advisable to prescribe which of the following?

    A. Ciprofloxacin
    B. Amoxicillin
    C. Cephalexin
    D. Doxycycline
    E. Sulfamethoxazole

13. What is the most likely mechanism of resistance for methicillin-resistant *Staphylococcus aureus* to antistaph penicillins?

    A. Methylation of the binding site
    B. Active efflux of the drug from the bacteria
    C. β-lactamase production
    D. Phosphorylation of the drug by bacterial enzymes
    E. Structural modifications of PBPs

14. Highly active antiretroviral therapy (HAART) in HIV infection is associated with which of the following?

    A. A decrease in viral mRNA copies/mL of blood
    B. A decrease in the rate of emergence of drug resistance
    C. A possible increase in CD4 cell count
    D. A reduced incidence of opportunistic infections
    E. All of the above

15. Oseltamivir and zanamivir are available for treatment of infections due to influenza A and B. The mechanism of their antiviral action is inhibition of which of the following?

    A. RNA polymerase
    B. Reverse transcriptase
    C. Thymidine kinase
    D. Neuraminidase
    E. Aspartate protease

16. In a patient who has an established hypersensitivity to metronidazole, what is the most appropriate drug to use for the management of pseudomembranous colitis?

    A. Ampicillin
    B. Clindamycin
    C. Doxycycline
    D. Ofloxacin
    E. Vancomycin

17. An AIDS patient who is being treated with multiple drugs, including AZT, lamivudine, indinavir, ketoconazole, and cotrimoxazole, develops breast hypertrophy, central adiposity, hyperlipidemia, insulin resistance, and nephrolithiasis. If these changes are related to his drug treatment, which of the following is the most likely cause?

    A. Azidothymidine
    B. Indinavir
    C. Ketoconazole
    D. Sulfamethoxazole
    E. Trimethoprim

18. Which one of the following drugs is most suitable in an immunocompromised patient for prophylaxis against infection due to *Cryptococcus neoformans?*

    A. Amphotericin B
    B. Ampicillin
    C. Fluconazole
    D. Nystatin
    E. Flucytosine

19. Which one of the following drugs is most likely to be associated with elevations of pancreatic enzymes, including amylase and lipase?

    A. Erythromycin
    B. Didanosine
    C. Isoniazid
    D. Zidovudine
    E. Pyrazinamide

20. The major mechanism of HSV resistance to acyclovir is

    A. a structural change in viral thymidine kinase
    B. a mutation in the gene that encodes DNA polymerase
    C. the loss of ability to produce viral thymidine kinase
    D. changes in reverse transcriptase
    E. mutations in the gene that codes for phosphotransferase

21. Despite its "age," penicillin G remains the drug of choice in the treatment of infections caused by which of the following organisms?

    A. *B. fragilis*
    B. *T. pallidum*
    C. *H. influenzae*
    D. *E. coli*
    E. *S. aureus*

22. Which one of the following drugs is most likely to be equally effective in the treatment of amebic dysentery and "backpacker's diarrhea"?

    A. Ciprofloxacin
    B. Diloxanide
    C. Metronidazole
    D. Quinacrine
    E. Trimethoprim-sulfamethoxazole

ANSWERS AND EXPLANATIONS

1. **Answer: D.** Life-threatening invasive aspergillosis, with necrotizing pneumonia, most commonly occurs in severely immunocompromised patients. The mortality rate approaches 50%, but high intravenous doses of amphotericin B may be lifesaving. Intravenous amphotericin B causes infusion-related hypotension (via histamine release), fever, and chills, which may be attenuated by the prior administration of NSAIDs and antihistamines. Adrenal steroids may provide supplementary stress support. Amphotericin B binds to ergosterol in fungal membranes, opening pores and disrupting membrane permeability.

2. **Answer: E.** For antitubercular activity, isoniazid (INH) must first be metabolically activated via a catalase present in mycobacteria. A decrease in expression of the *cat* G gene that encodes this enzyme is the mechanism of high-level resistance to INH.

3. **Answer: A.** Azithromycin is highly effective as an oral agent in the management of pharyngitis caused by gram-positive cocci and may necessitate only a short course of therapy. In patients who have marked hypersensitivity to penicillins, it is inappropriate to use a cephalosporin, even though cefaclor is active against common oropharyngeal pathogens. Doxycycline should not be used in children. One must assume that complete cross-allergenicity exists between different members of the penicillin class of antibiotics, and, in any case, penicillin G is not usually given orally because of its lability in gastric acid. Vancomycin would need parenteral administration, and this antibiotic should be reserved for more serious bacterial infections.

4. **Answer: C.** Organisms associated with sexually transmitted diseases include chlamydia, *Neisseria gonorrhea, Treponema* (syphilis), *Trichomonas,* and *Gardnerella vaginalis.* The latter two organisms are effectively treated with the drug metronidazole. Metronidazole has a chemical structure that results in a disulfiram-like effect on aldehyde dehydrogenase, causing reactions with ethanol. Patients should be cautioned not to consume alcoholic beverages while on this drug.

5. **Answer: B.** Ceftriaxone is eliminated largely via biliary excretion, and decreases in renal function do not usually require a dose reduction. All of the other antimicrobial drugs listed are eliminated by the kidney, at rates proportional to creatinine clearance, so major dose reductions would be needed in patients with renal dysfunction to avoid toxicity.

6. **Answer: B.** Mebendazole is the drug of choice for treatment of all nematode infections (hookworm, roundworm, pinworm, whipworm). Pyrantel is considered equally effective as mebendazole for nematodes. Praziquantel is used for tapeworms (cestodes) and flukes (trematodes).

7. **Answer: A.** Macrolides (azithromycin) are effective for common causes of pneumonias such as Strep pneumonia, Haemophilus influenza, Mycoplasma, Legionella, and Chlamydophila. The drugs work at the 50S ribosomal subunit to inhibit translocation of the peptidyl tRNA from the acceptor to the donor site.

8. **Answer: B.** The most common pathogens implicated in bacterial meningitis in a neonate (age <1 month) are group B streptococci, followed by *E. coli*. Meningococci and pneumococci become prevalent after 1 month of age, and *H. influenzae* is becoming rarer since the availability of a vaccine. A third-generation cephalosporin (e.g., cefotaxime) would be administered because it provides coverage for most of the organisms mentioned. However, ampicillin is also needed to cover for *Listeria monocytogenes*, which occurs with an incidence of 7 to 8% in neonatal meningitis.

9. **Answer: A.** Clindamycin has a mechanism of action similar to, if not identical with, erythromycin and related macrolides. They bind to rRNA bases on the 50S subunit to prevent translocation of peptidyl-mRNA from the acceptor to the donor site. Chloramphenicol also binds to the 50S subunit but interferes with the activity of peptidyltransferase. Gentamicin and tetracyclines bind to the 30S ribosomal subunit. Imipenem is a cell-wall synthesis inhibitor, acting similarly to beta-lactams.

10. **Answer: A.** Microbial resistance to fluoroquinolones is increasing, and some strains of *Streptococcus pneumoniae* are now resistant to ciprofloxacin. The mechanism can involve changes in the structure of topoisomerase IV, one of the "targets" of fluoroquinolones, which inhibit nucleic acid synthesis. Pneumococcal resistance to penicillins is also increasing via changes in penicillin-binding proteins (PBPs). The other mechanisms listed underlie microbial resistance to other antibiotics as follows: sulfonamides **(choice B),** macrolides (**choice C**), extended-spectrum penicillins (**choice D**), and beta-lactams (**choice E**).

11. **Answer: B.** Aminoglycosides like gentamicin work on aerobic gram negative rods. They require oxygen to enter bacteria, and, as such, do not treat any anaerobes including *Bacteroides fragilis*. They can be used with penicillins such as ampicillin against *Listeria* for a synergistic effect.

12. **Answer: E.** Drugs that cause oxidative stress may precipitate acute hemolysis in patients who lack G6PD because they have a limited ability to generate NADPH, which restricts the formation of glutathione. Drugs in this category include primaquine, quinine, nitrofurantoin, sulfonamides, and TMP-SMX.

13. **Answer: E.** Antistaph penicillins are inherently resistant to cleavage by bacterial beta-lactamases. Instead, resistance develops when the target for these drug, PBPs, are altered such that the drug doesn't bind effectively.

14. **Answer: E.** HAART in the management of HIV infection is reported in many but not all patients to decrease viral load, increase CD4 cells, slow disease progression, and reduce opportunistic infections. However, in terms of the chemotherapy of AIDS, the word *cure* has little meaning. Discontinuance of HAART, after suppression of viral RNA copies below the sensitivity of the best current methods of analysis, is followed by the reemergence of detectable viral RNA in the blood within a few months.

15. **Answer: D.** Neuraminidase is an enzyme on the lipid envelope of influenza A and B virions that prevents their clumping together and also their binding to the surface of cells that have been already infected. Neuraminidase inhibitors interfere with this activity and reduce the availability of virions for entry into noninfected cells. Oseltamivir and zanamivir decrease the severity and duration of symptoms if given within a day or two of onset.

16. **Answer: E.** Vancomycin is usually considered to be a backup drug to metronidazole in colitis due to *Clostridium difficile* on the grounds that it is no more effective, is more costly, and should be reserved for treatment of resistant gram-positive coccal infections. None of the other drugs has activity in pseudomembranous colitis—indeed, they may cause it!

17. **Answer: B.** AIDS patients being treated with protease inhibitors (e.g., indinavir) have developed a syndrome involving derangement of lipid and CHO metabolism. Changes in lipid metabolism and distribution occur quite commonly, and type 2 diabetes has also been reported. Indinavir is also notable for its tendency to precipitate in the urinary tract, causing nephrolithiasis, unless the patient is maintained in a high state of hydration.

18. **Answer: C.** Fluconazole is distinctive in terms of its ability to penetrate into the cerebrospinal fluid, reaching levels similar to those in the blood. It is effective against *C. neoformans* and has become the most appropriate drug to use in both prophylaxis and suppression because of its oral efficacy and low toxicity compared with amphotericin B. Flucytosine is also active against *C. neoformans* but is not used alone because of rapid emergence of resistance. Nystatin is too toxic for systemic use.

19. **Answer: B.** Pancreatic dysfunction, heralded by large increases in serum amylase and lipase, is associated with the use of several reverse-transcriptase inhibitors (RTIs). Didanosine appears to be the worst offender, and pancreatitis is the most characteristic adverse effect of this particular NRTI. Conditions enhancing susceptibility to drug-induced pancreatic dysfunction include hypertriglyceridemia, hypercalcemia, and history of excessive ethanol use. Liver dysfunction including hepatitis may occur with the antitubercular drugs, isoniazid, and pyrazinamide. Cholestasis is associated with the estolate form of erythromycin.

20. **Answer: C.** To inhibit DNA polymerases in HSV, acyclovir must undergo initial monophosphorylation by a viral specific thymidine kinase (TK). Most HSV strains resistant to acyclovir lack this enzyme and are thus TK^- strains. A few strains of HSV are resistant to acyclovir by structural changes in TK that lower substrate affinity or by mutations in the gene that encode viral DNA polymerases.

21. **Answer: B.** Indications for the use of penicillin G are currently limited for a number of reasons. The drug has a narrow spectrum, is susceptible to beta-lactamases, and may cause hypersensitivity. Also, alternative antibiotics are available. However, penicillin G remains the drug of choice in syphilis, usually given IM as benzathine penicillin G, but as the Na^+ or K^+ salt IV in neurosyphilis. What would you do for patients who are highly allergic to penicillins? (Consider tetracyclines, or possibly desensitization.)

22. **Answer: C.** In amebic dysentery caused by *Entamoeba histolytica* and gastrointestinal infections with diarrhea (“backpacker’s diarrhea”) due to *Giardia lamblia,* metronidazole is the drug of choice. Diloxanide is a backup drug for noninvasive intestinal amebiasis, but it has minimal activity in *Giardia* infections. Quinacrine has effectiveness in giardiasis but not amebiasis. TMP-SMX has antiprotozoal effectiveness in *Pneumocystis jiroveci,* pneumonia. Ciprofloxacin is devoid of antiprotozoal activity.

PART VI

Drugs for Inflammatory and Related Disorders

Histamine and Antihistamines 1

Learning Objectives

- ❑ Answer questions about histamine
- ❑ Use knowledge of H1 antagonists to describe their appropriate use

HISTAMINE

Histamine is an autacoid present at high levels in the lungs, skin, and gastrointestinal tract. It is released from mast cells and basophils by type I hypersensitivity reactions, drugs, venoms, and trauma.

- Histamine receptors are of the serpentine family, with 7 transmembrane–spanning domains with G-protein–coupled second messenger effectors.
  - H_1 activation
    - ↑ capillary dilation (via NO) →↓ BP
    - ↑ capillary permeability →↑ edema
    - ↑ bronchiolar smooth muscle contraction (via IP_3 and DAG release)
    - ↑ activation of peripheral nociceptive receptors →↑ pain and pruritus
    - ↓ AV nodal conduction
  - H_2 activation
    - ↑ gastric acid secretion →↑ gastrointestinal ulcers
    - ↑ SA nodal rate, positive inotropism, and automaticity

H_1 Antagonists

High-Yield

- Mechanism of action:
  - H_1 antagonists act as competitive antagonists of histamine and therefore may be ineffective at high levels of histamine.
  - Vary in terms of both pharmacologic and kinetic properties, but all require hepatic metabolism and most cross the placental barrier.

Table VI-1-1. Properties of Major Antihistamines

Drug	M Block	Sedation	Antimotion	Other Characteristics
Diphenhydramine	+++	+++	+++	Widely used OTC drug
Promethazine	+++	+++	++	Some α block and local anesthetic action
Chlorpheniramine	++	++	++	Possible CNS stimulation
Meclizine	++	++	++++	Highly effective in motion sickness
Cetirizine	+/−	+	0	
Loratadine	+/−	0	0	No CNS entry
Fexofenadine	+/−	0	0	No CNS entry

- Uses:
  - Allergic reactions: hay fever, rhinitis, urticaria
  - Motion sickness, vertigo
  - Nausea and vomiting with pregnancy
  - Preoperative sedation
  - OTC: sleep aids and cold medications
  - Acute EPSs
- Side effects: extensions of M block and sedation (additive with other CNS depressants)

Drugs Used in Gastrointestinal Dysfunction

2

Learning Objectives

- ❑ Solve problems concerning drugs used in peptic ulcer disease
- ❑ Differentiate between H2 antagonists and PPIs
- ❑ Solve problems concerning antacids: $Al(OH)_3$, $Mg(OH)_2$, $CaCO_3$
- ❑ Describe mechanism of action, side effects, and appropriate use of misoprostol, sucralfate, and bismuth subsalicylate
- ❑ Answer questions about antiemetics

DRUGS USED IN PEPTIC ULCER DISEASE (PUD)

Drug Mechanisms

High-Yield

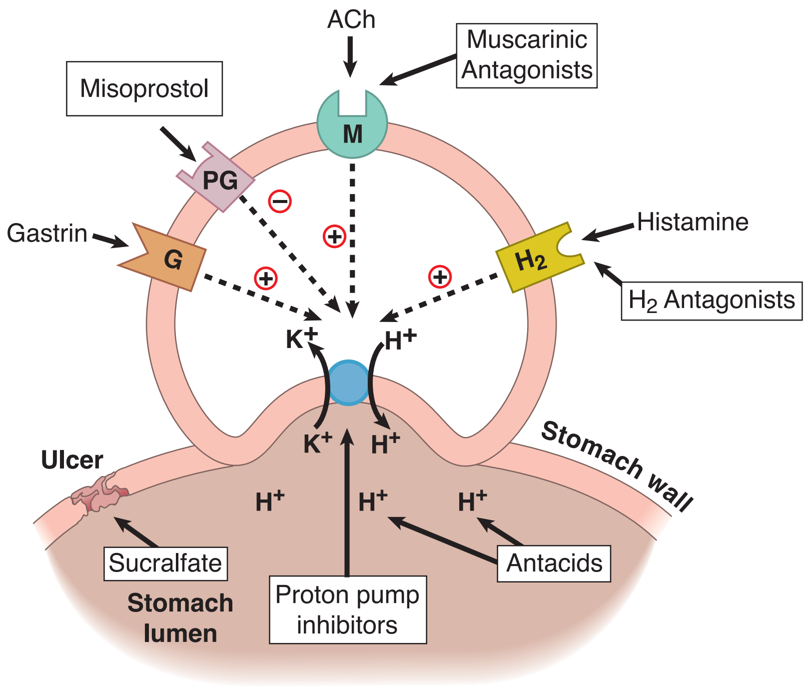


Figure VI-2-1. Drug Actions in PUD

H_2 Antagonists (Cimetidine, Ranitidine, Famotidine)

- Mechanisms of action:
  - Suppress secretory responses to food stimulation and nocturnal secretion of gastric acid via their ability to decrease (indirectly) the activity of the proton pump.
  - Also partially antagonize HCl secretion caused by vagally or gastrin-induced release of histamine from ECL-like cells (GI mast cells)
  - No effects on gastric emptying time
- Uses:
  - PUD (overall less effective than proton pump inhibitors)
  - Gastroesophageal reflux disease (GERD)
  - Zollinger-Ellison syndrome
- Side effects:
  - Cimetidine is a major inhibitor of P450 isoforms → drug interaction via ↑ effects
  - Cimetidine →↓ androgens → gynecomastia and ↓ libido

Proton Pump Inhibitors

- Mechanism of action:
  - **Omeprazole** and related "–prazoles" are irreversible, direct inhibitors of the proton pump (K^+/H^+ antiport) in the gastric parietal cell
- Uses:
  - More effective than H_2 blockers in peptic ulcer disease (PUD)
  - Also effective in GERD and Zollinger-Ellison syndrome
  - Eradication regimen for *H. pylori*

Misoprostol

- Mechanism of action: PGE_1 analog, which is cytoprotective →↑ mucus and bicarbonate secretion and ↓ HCl secretion
- Uses: Previously for NSAID-induced ulcers, but PPIs are now used

Sucralfate

- Mechanism of action: polymerizes on gastrointestinal luminal surface to form a protective gel-like coating of ulcer beds. Requires acid pH (antacids may interfere)
- Uses: ↑ healing and ↓ ulcer recurrence

Bismuth Subsalicylate

- Mechanism of action: like sucralfate, binds selectively to ulcer, coating it, and protecting it from acid and pepsin
- Combined with metronidazole and tetracycline to eradicate *H. pylori* (BMT regimen)

Antacids: $Al(OH)_3$, $Mg(OH)_2$, $CaCO_3$

- Mechanism of action: bases that neutralize protons in the gut lumen
- Side effects: Constipation (Al^{+++}), diarrhea (Mg^{++}); rebound hyperacidity

Clinical Correlate

Antacids and Drug Absorption

- ↑ oral absorption of weak bases (e.g., quinidine)
- ↓ oral absorption of weak acids (e.g., warfarin)
- ↓ oral absorption of tetracyclines (via chelation)

ANTIEMETICS

Antiemetic Mechanisms

High-Yield

The figure below shows the complexity of the emetic pathways with an impact on the vomiting center and reveals the multiplicity of receptor types involved, including those activated by ACh, DA, 5HT, histamine, and endogenous opiopeptides.

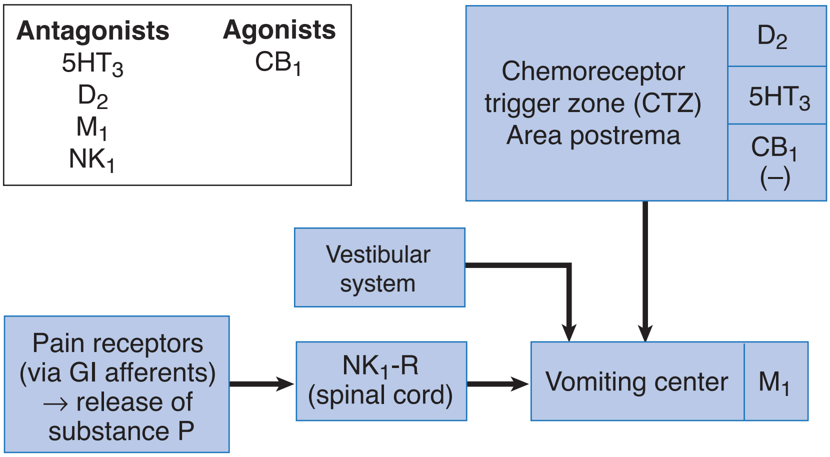


Figure VI-2-2. Emetic Pathways and Drug Action

Drugs for Nausea and Vomiting

Drugs for nausea and vomiting include:

- $5HT_3$ (a serotonin receptor: *see* next chapter) antagonists: ondansetron (commonly used in cancer chemotherapy), granisetron
- DA antagonists: prochlorperazine, metoclopramide (also used in cancer chemotherapy; also prokinetic in GERD)
- H_1 antagonists: diphenhydramine, meclizine, promethazine
- Muscarinic antagonists: scopolamine
- Cannabinoids: dronabinol
- NK_1-receptor antagonist: aprepitant (NK_1 is a receptor to substance P)

Clinical Correlate

Opioid analgesics (e.g., morphine) have duality of action: ↓ emesis by activating receptors that decrease pain transmission and ↑ emesis by activating receptors in the CTZ.

Drugs Acting on Serotonergic Systems

3

Learning Objectives

- ❏ Demonstrate understanding of drug actions on 5HT receptors
- ❏ Describe treatment options for migraine headaches

- Serotonin (5-hydroxytryptamine, 5HT) is an autacoid synthesized and stored in gastrointestinal cells, neurons, and platelets. Metabolized by MAO type A, its metabolite 5-hydroxyinolacetic acid (5HIAA) is a marker for carcinoid.
- Of the 7 receptor subtype families, all are G-protein coupled except $5HT_3$, which is coupled directly to an ion channel.

DRUG ACTIONS ON 5HT RECEPTORS

$5HT_{1(a–h)}$

- Found in CNS (usually inhibitory) and smooth muscle (excitatory or inhibitory)
- Drug: **buspirone**
  - Partial agonist at $5HT_{1a}$ receptors → anxiolytic (generalized anxiety disorder [GAD])
- Drug: **sumatriptan and other triptans**
  - Agonist at $5HT_{1d}$ receptors in cerebral vessels →↓ migraine pain
  - Side effects of "–triptans": possible asthenia, chest or throat pressure or pain

$5HT_{2(a–c)}$

- Found in CNS (excitatory)
- In periphery, activation → vasodilation, contraction of gastrointestinal, bronchial, and uterine smooth muscle, and platelet aggregation

- Drugs:
  - **Olanzapine** and other atypical antipsychotics: antagonist at $5HT_{2a}$ receptors in CNS →↓ symptoms of psychosis
  - **Cyproheptadine**
    - $5HT_2$ antagonist used in carcinoid, other gastrointestinal tumors, and postgastrectomy; also used for anorexia nervosa; serotonin syndrome
    - Has marked H_1-blocking action: used in seasonal allergies

$5HT_3$

- Found in area postrema, peripheral sensory and enteric nerves
- Mechanism of action: activation opens ion channels (no second messengers)
- Drugs: **ondansetron** and "–setrons": antagonists →↓ emesis in chemotherapy and radiation and postoperatively

DRUGS USED IN MIGRAINE HEADACHES

Sumatriptans and other triptans are agonists at $5HT_{1d}$ receptors and used for acute migraine treatment.

Note

Ergonovine

- Mechanism of action: uterine smooth muscle contraction
- Use: given intramuscularly after placental delivery

Ergot Alkaloids

- **Ergotamine**
  - Mechanism of action: acts as partial agonist at both α and $5HT_2$ receptors in the vasculature and possibly in CNS; vasoconstrictive actions to decrease pulsation in cerebral vessels may be relevant to acute actions of ergotamine during migraine attack
  - Uses: acute attacks
  - Side effects: gastrointestinal distress, prolonged vasoconstriction → ischemia and gangrene, abortion near term

Other Medications

In addition to the "–triptans" and ergots:

- Analgesics: ASA (+/– caffeine, or butabarbital), other NSAIDs, acetaminophen (+/– caffeine), oral or injectable opioid-analgesics, and butorphanol (spray)
- Prophylaxis: propranolol, topiramate, valproic acid

Eicosanoid Pharmacology 4

Learning Objectives

- ❏ Demonstrate understanding of NSAIDs
- ❏ Differentiate leukotrienes, prostaglandins, and thromboxanes

EICOSANOIDS

Eicosanoids are cell-regulating polyunsaturated fatty acids primarily synthesized from arachidonic acid and released by the action of phospholipase A_2 from lipids in cell membranes.

- Are present in low concentrations in most cells but are synthesized and released "on demand" in response to stimuli, including IgE-mediated reactions, inflammatory mediators, trauma, heat, and toxins
- Interact with specific receptors, which are G-proteins coupled to second messenger effector systems

Bridge to Physiology

Prostaglandins (PGs) are cytoprotective in the stomach, dilate renal vasculature, contract the uterus, and maintain the ductus arteriosus. Thromboxane (TxA_2) causes platelet aggregation. GI PGs and platelets TxA_2s are synthesized by COX 1 (constitutive). COX 2 (inducible) synthesizes PGs involved in inflammation, fever, and pain. Both enzymes synthesize renal PGs →↑ RBF.

Eicosanoid Mechanisms

High-Yield

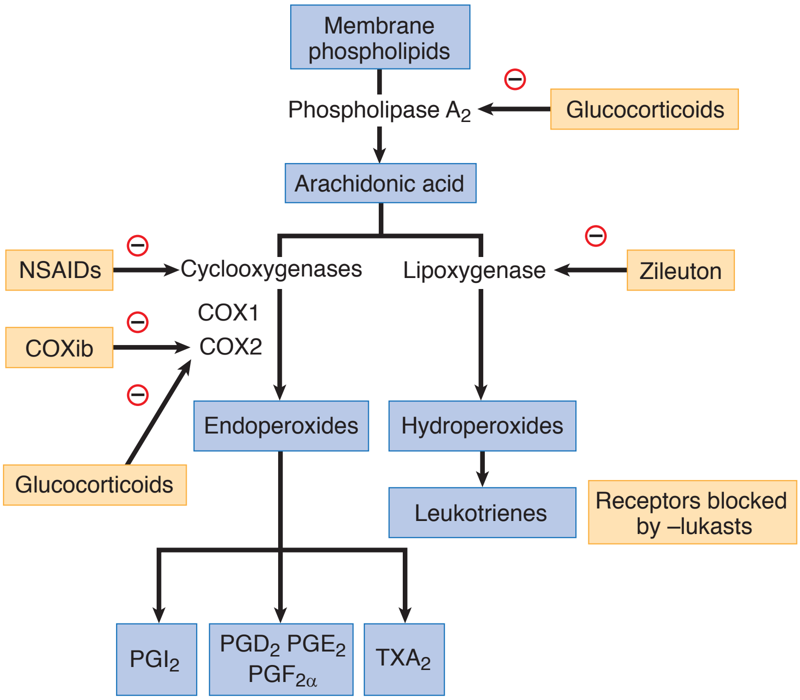


Figure VI-4-1. Drugs Acting on Eicosanoids

Leukotrienes (LTs)

Leukotrienes (LTs) are formed (via hydroperoxides) from the action of lipoxygenases on arachidonic acid.

- LTB_4:
  - Mechanism of action: inflammatory mediator → neutrophil chemoattractant; activates PMNs; ↑ free radical formation → cell damage
- LTA_4, LTC_4, and LTD_4
  - Cause anaphylaxis and bronchoconstriction (role in asthma)

Leukotrienes are "targets" for the following:

- Glucocorticoids: →↓ phospholipase A_2 activity → contributes to both antiinflammatory and immunosuppressive actions
- Zileuton: inhibits lipoxygenase →↓ LTs and is used in treatment of asthma
- Zafirlukast and "–lukasts": LT-receptor antagonists used in treatment of asthma

Prostaglandins

Prostaglandins (PGs) are formed (via endoperoxides) from the actions of cyclooxygenases (COXs).

- COX 1 is expressed in most tissues, including platelets and stomach, where it acts to synthesize thromboxane and cytoprotective prostaglandins, respectively.
- COX 2 is expressed in the brain and kidney and at sites of inflammation.

Note

Indomethacin is used to close a patent ductus arteriosus.

PGE_1

- Drugs:
  - **Misoprostol** used previously in treatment of NSAID-induced ulcers (protective action on gastric mucosa)
  - **Alprostadil**
    - Maintains patency of ductus arteriosus
    - Vasodilation; used in male impotence
- Contraindicated in pregnancy, unless used as an abortifacient (misoprostol in combination with mifepristone)

PGE_2

- Mechanism of action: uterine smooth muscle contraction
- Uses: **dinoprostone** can be used for "cervical ripening" and as abortifacient

$PGF_2\alpha$

- Mechanism of action: uterine and bronchiolar smooth muscle contraction
- Drugs:
  - **Carboprost** used as abortifacient
  - **Latanoprost** for treatment of glaucoma (↓ intraocular pressure)

PGI_2 (Prostacyclin)

- Platelet stabilizer and vasodilator
- Drug: **epoprostenol**
- Uses: pulmonary hypertension

PGE_2 and PGF_2

- Both ↑ in primary dysmenorrhea
- Therapeutic effects of NSAIDs may be due to inhibition of their synthesis

Thromboxanes (TXAs)

TXA_2

- Platelet aggregator (inhibition of synthesis underlies protective role of acetylsalicylic acid [ASA] post-MI)

NONSTEROIDAL ANTIINFLAMMATORY DRUGS

Most nonsteroidal antiinflammatory drugs (NSAIDs) are nonselective inhibitors of cyclooxygenases, acting on both COX 1 and COX 2 isoforms to decrease formation of PGs and thromboxanes.

- Are analgesic, antipyretic, and antiinflammatory
- Have antiplatelet effects
- Acetylsalicylic acid (ASA) is prototype of the group, which includes more than 20 individual drugs

Acetylsalicylic Acid (ASA; Aspirin)

High-Yield

- Causes irreversible inhibition of COX
- Covalent bond via acetylation of a serine hydroxyl group near the active site
- Actions are dose-dependent:
  - **Antiplatelet aggregation**. Low dose, the basis for post-MI prophylaxis and to reduce the risk of recurrent TIAs
  - **Analgesia and antipyresis**. Moderate dose

Bridge to Physiology and Biochemistry

Platelet Stability and Eicosanoids

Activation of TxA_2 receptors → stimulation of phospholipase C →↑ PIP_2 hydrolysis →↑ IP_3→ mobilization of bound Ca^{2+}→↑ free Ca^{2+}→ platelet aggregation.

Activation of PGI_2 receptors → stimulation of adenylyl cyclase →↑ cAMP →↑ activity of internal Ca^{2+} "pumps" →↓ free Ca^{2+}→ platelet stabilization.

- **Antiinflammatory**. High doses
- **Uric acid elimination**
  - Low to moderate doses: ↓ tubular secretion → hyperuricemia
  - High doses: ↓ tubular reabsorption → uricosuria
- **Acid-base and electrolyte balance**
  - Dose-dependent actions
  - High therapeutic: mild uncoupling of oxidative phosphorylation →↑ respiration →↓ pCO_2→**respiratory alkalosis**→ renal compensation →↑ HCO_3^- elimination →**compensated** respiratory alkalosis (pH = normal, ↓ HCO_3^-, ↓ pCO_2)
  - In adults, this can be a stable condition; in children →↑ toxicity.
  - Toxic doses: inhibits respiratory center →↓ respiration →↑ pCO_2→**respiratory acidosis** (↓ pH, ↓ HCO_3^-, normalization of pCO_2) plus inhibition of Krebs cycle and severe uncoupling of oxidative phosphorylation (↓ ATP) →**metabolic acidosis**, hyperthermia, and hypokalemia (↓ K^+).

• Side effects:
- Gastrointestinal irritation: gastritis, ulcers, bleeding
- Salicylism: tinnitus, vertigo, ↓ hearing—often first signs of toxicity
- Bronchoconstriction: exacerbation of asthma
- Hypersensitivity, especially the “triad” of asthma, nasal polyps, rhinitis
- Reye syndrome: encephalopathy
- ↑ bleeding time (antiplatelet)
- Chronic use: associated with renal dysfunction
- Drug interactions: ethanol (↑ gastrointestinal bleeding), OSUs and warfarin (↑ effects), and uricosurics (↓ effects)

• Aspirin overdose and management:
- Extensions of the toxic actions described above, plus at high doses vasomotor collapse occurs, with both respiratory and renal failure.
- No specific antidote. Management includes gastric lavage (+/− activated charcoal) plus ventilatory support and symptomatic management of acid-base and electrolyte imbalance, and the hyperthermia and resulting dehydration. Increased urine volume and its alkalinization facilitate salicylate renal elimination. (Note: ASA follows zero-order elimination kinetics at toxic doses.)

Other NSAIDs

Types

- Reversible inhibitors of COX 1 and COX 2, with analgesic, antipyretic, and antiinflammatory actions, include:
  - Ibuprofen
  - Naproxen
  - Indomethacin
  - Ketorolac
  - Sulindac
- Comparisons with ASA:
  - Analgesia: ketorolac > ibuprofen/naproxen > ASA
  - Gastrointestinal irritation: < ASA, but still occurs (consider misoprostol)
  - Minimal effects on acid-base balance; no effects on uric acid elimination
  - Allergy:common, possible cross-hypersensitivity with ASA
  - Renal: chronic use may cause nephritis, nephritic syndrome, acute failure (via ↓ formation of PGE_2 and PGI_2, which normally maintain GFR and RBF)—does not occur with sulindac
- Specific toxicities:
  - Indomethacin: thrombocytopenia, agranulocytosis, and > CNS effects
  - Sulindac: Stevens-Johnson syndrome, hematotoxicity

Clinical Correlate

NSAIDs are associated with an increased risk of adverse cardiovascular thrombotic events such as MI and stroke.

Selective COX 2 Inhibitors: Celecoxib

- Compared with conventional NSAIDs, it is no more effective as an antiinflammatory agent.
- Primary differences are:
  - Less gastrointestinal toxicity
  - Less antiplatelet action
- However, it may possibly exert prothrombotic effects via inhibition of endothelial cell function (MI and strokes).
- Cross-hypersensitivity between celecoxib and sulfonamides

Recall Question

Which of the following drugs is used in the management of pulmonary hypertension?

A. Alprostadil

B. Dinoprostone

C. Epoprostenol

D. Latanoprost

Answer: C

OTHER DRUGS

Clinical Correlate

"Tot" Toxicity

Young children are gustatory explorers. Among the compounds responsible for toxicity in children age <3 are common household items: aspirin, acetaminophen (people know about Reye syndrome!), and supplementary iron tablets.

Acetaminophen

High-Yield

- Mechanisms:
  - No inhibition of COX in peripheral tissues and lacks significant antiinflammatory effects
  - Equivalent analgesic and antipyretic activity to ASA due to inhibition of cyclooxygenases in the CNS
- Comparisons with ASA:
  - No antiplatelet action
  - Not implicated in Reye syndrome
  - No effects on uric acid
  - Not bronchospastic (safe in NSAID hypersensitivity and asthmatics)
  - Gastrointestinal distress is minimal at low to moderate doses
- Overdose and management:
  - Hepatotoxicity—Acetaminophen is metabolized mainly by liver glucuronyl transferase to form the inactive conjugate. A minor pathway (via P450) results in formation of a reactive metabolite (*N*-acetylbenzoquinoneimine), which is inactivated by glutathione (GSH). In overdose situations, the finite stores of GSH are depleted. Once this happens, the metabolite reacts with hepatocytes, causing nausea and vomiting, abdominal pain, and ultimately liver failure due to centrilobular necrosis. Chronic use of ethanol enhances liver toxicity via induction of P450.
  - Management of the hepatotoxicity: *N*-acetylcysteine (supplies –SH groups), preferably within the first 12 hours (*N*-acetylcysteine is also used as a mucolytic for cystic fibrosis)

Drugs Used for Treatment of Rheumatoid Arthritis

5

Learning Objectives

- ❑ Describe drug therapy for rheumatoid arthritis that potentially slows disease progression and avoids side effects of NSAIDs

RHEUMATOID ARTHRITIS

Treatment Strategies

NSAIDs are commonly used in the initial management of rheumatoid arthritis (RA), but the doses required generally result in marked adverse effects. NSAIDs decrease pain and swelling but have no beneficial effect on the course of the disease or bone deterioration.

- DMARDs are thought to slow disease progression.
- DMARDs may be started with NSAIDs at the time of initial diagnosis if symptoms are severe because DMARDs take 2 weeks to 6 months to work.
- Hydroxychloroquine is often recommended for mild arthritis and methotrexate (MTX) for moderate to severe RA.
- Other DMARDs are used less frequently, sometimes in combination regimens for refractory cases.

DMARD Mechanisms

High-Yield

Table VI-5-1. Disease-Modifying Antirheumatic Drugs (DMARDs)

Drug	Mechanism(s)	Side Effects
Hydroxychloro-quine	Stabilizes lysosomes and ↓ chemotaxis	GI distress and visual dysfunction (cinchonism), hemolysis in G6PD deficiency
Methotrexate	Cytotoxic to lymphocytes	Hematotoxicity, hepatotoxicity
Sulfasalazine	Sulfapyridine →↓ B-cell functions; 5-ASA possibly inhibits COX	Hemolysis in G6PD deficiency
Glucocorticoids	↓ LTs, ILs, and platelet-activating factor (PAF)	ACTH suppression, cushingoid state, osteoporosis, GI distress, glaucoma
Leflunomide	Inhibits dihydro-orotic acid dehydrogenase (DHOD) →↓ UMP →↓ ribonucleotides → arrests lymphocytes in G_1	Alopecia, rash, diarrhea, hepatotoxicity
Etanercept	Binds tumor necrosis factor (TNF); is a recombinant form of TNF receptor	Infections
Infliximab, adalimumab	Monoclonal antibody to TNF	Infections
Anakinra	IL-1 receptor antagonist	Infections

Drugs Used for Treatment of Gout 6

Learning Objectives

❑ Demonstrate understanding of prophylaxis of chronic gout and treatment of acute inflammatory episodes

GOUT

Acute Inflammatory Episodes

High-Yield

NSAIDs are used as initial therapy for acute gout attacks; colchicine and intra-articular steroids are alternatives.

- Colchicine
  - Mechanism of action: binds to tubulin →↓ microtubular polymerization, ↓ LTB_4, and ↓ leukocyte and granulocyte migration
  - Side effects: diarrhea and gastrointestinal pain (acute); hematuria, alopecia, myelosuppression, gastritis, and peripheral neuropathy (longer use)

Chronic Gout

High-Yield

- Drug strategy (**prophylaxis**): reduction of uric acid pool
- Allopurinol and febuxostat
  - Mechanism: inhibit xanthine oxidase →↓ purine metabolism →↓ uric acid (also useful in cancer chemotherapy and radiation)
  - Side effects: rash, hypo[xanthine] stones
  - Drug interactions: inhibits 6-mercaptopurine (6-MP) metabolism

Clinical Correlate

Rasburicase is a recombinant urate-oxidase enzyme for the prevention of tumor lysis syndrome. This drug rapidly reduces serum uric acid; by contrast, the action of allopurinol and febuxostat is to decrease uric acid formation.

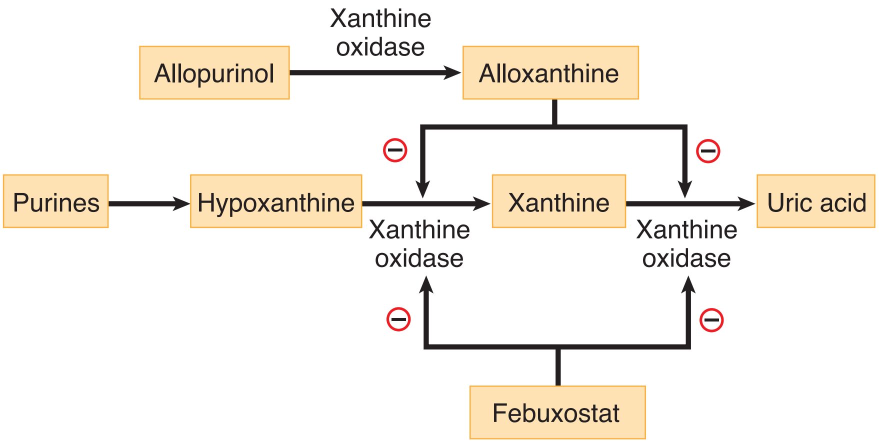


Figure VI-6-1. Mechanism of Action of Allopurinol

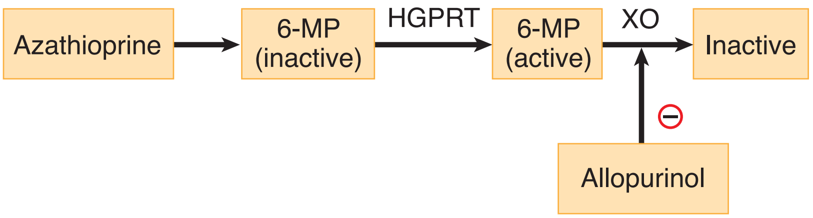


Figure VI-6-2. Drug Interaction between Allopurinol and 6-Mercaptopurine

- Pegloticase
  - Mechanism: recombinant urate-oxidase enzyme for refractory gout; metabolizes uric acid to allantoin →↓ plasma uric acid
  - Side effects: anaphylaxis, urticaria
- Probenecid
  - Mechanism: inhibits proximal tubular reabsorption of urate, but ineffective if GFR <50 mL/min
  - Drug interactions: inhibits secretion of weak acid drugs such as penicillins, cephalosporins, and fluoroquinolones

Glucocorticoids 7

Learning Objectives

❑ Describe mechanism of action and adverse effects of commonly used glucocorticoid medications

GLUCOCORTICOID PROPERTIES

Table VI-7-1. Synthetic Derivatives of Cortisol

Drugs	Glucocorticoid Activity	Mineralocorticoid Activity	Duration
Cortisol, hydrocortisone	1	1	Short
Prednisone	4	0.3	Medium
Triamcinolone	5	0	Intermediate
Betamethasone	25	0	Long-acting
Dexamethasone	30	0	Long-acting

- Mechanisms of action:
  - Cellular effects
    - ↓ leukocyte migration
    - ↑ lysosomal membrane stability →↓ phagocytosis
    - ↓ capillary permeability
  - Biochemical actions
    - Inhibit PLA_2 (via lipocortin expression) →↓ PGs and ↓ LTs
    - ↓ expression of COX 2
    - ↓ platelet-activating factor
    - ↓ interleukins (e.g., IL-2)
- Uses: antiinflammatory and immunosuppressive
- Side effects:
  - Suppression of ACTH: cortical atrophy, malaise, myalgia, arthralgia, and fever; may result in a shock state with abrupt withdrawal
  - Iatrogenic cushingoid syndrome → fat deposition, muscle weakness/atrophy, bruising, acne

Clinical Correlate

Minimize Steroidal Toxicity

- Alternate-day therapy; local application (e.g., aerosols)
- Dose-tapering to avoid cortical suppression

- Hyperglycemia due to ↑ gluconeogenesis → increased insulin demand and other adverse effects
- Osteoporosis: vertebral fractures; aseptic hip necrosis
- ↑ gastrointestinal acid and pepsin release → ulcers, gastrointestinal bleeding
- Electrolyte imbalance: Na^+/water retention → edema and hypertension, hypokalemic alkalosis, hypocalcemia
- ↓ skeletal growth in children
- ↓ wound healing, ↑ infections (e.g., thrush)
- ↑ glaucoma, ↑ cataracts (via ↑ sorbitol)
- ↑ mental dysfunction

Drugs Used for Treatment of Asthma 8

Learning Objectives

- ❏ Describe the mechanism of action of beta-receptor agonists, muscarinic-receptor blockers, glucocorticoids, and anti-leukotrienes in asthma
- ❏ Compare the uses and side-effects of theophylline, cromolyn, and nedocromil

ASTHMA TREATMENTS

Asthma Overview

High-Yield

Asthma is an inflammatory disease associated with bronchial hyperreactivity (BHR), bronchospasm, increased mucus secretion, edema, and cellular infiltration.

- Early asthmatic responses (EAR) lasting 30–60 minutes are associated with bronchospasm from the actions of released histamine and leukotrienes.
- Late asthmatic responses (LAR) involve infiltration of eosinophils and lymphocytes into airways → bronchoconstriction and inflammation with mucous plugging.

Management of asthma includes bronchodilators to provide short-term relief and antiinflammatory agents to reduce bronchial hyperactivity and protect against cellular infiltration.

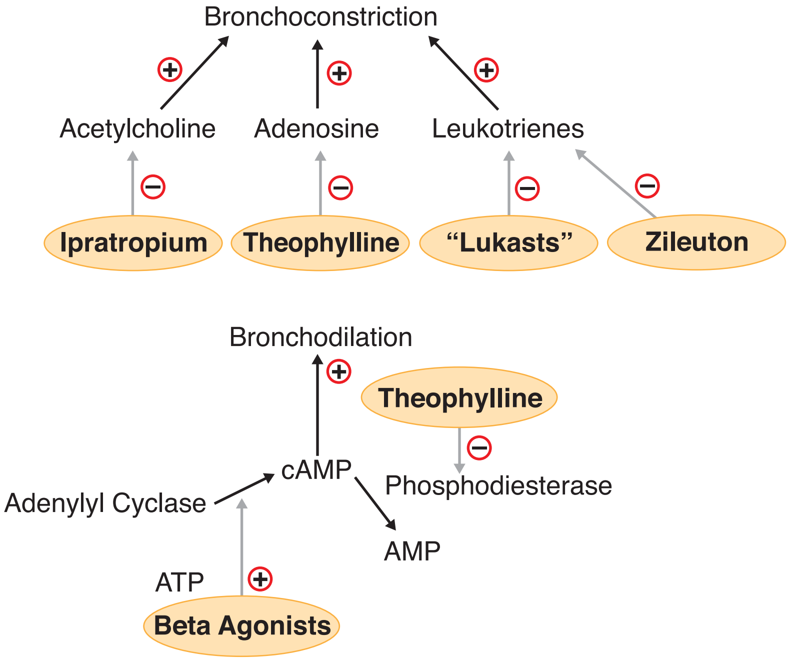


Figure VI-8-1. Drug Actions on Bronchiolar Smooth Muscle

Beta-Receptor Agonists

- Beta-2 selective drugs (albuterol, metaproterenol, terbutaline) are widely used for relief of acute bronchoconstriction and in prophylaxis of exercise-induced asthma (*see* Figure VI-8-1).
- Longer-acting drugs (e.g., salmeterol) may decrease nighttime attacks (prophylaxis only) and permit dosage reduction of other agents.
- Aerosolic forms have low potential for systemic toxicity but may cause anxiety, muscle tremors, and cardiovascular toxicity with overuse.

Muscarinic-Receptor Blockers

- Ipratropium and tiotropium used via inhalation cause bronchodilation in acute asthma, especially in COPD patients, and they may be safer than β agonists are in patients with cardiovascular disease.
- They are the drugs of choice in bronchospasm caused by β blockers.
- There are minor atropine-like effects.

Theophylline

- Bronchodilates via inhibition of phosphodiesterase (PDE) →↑ cAMP and also by antagonism of adenosine (a bronchoconstrictor)
- Mainly adjunctive; regular use may decrease symptoms, but narrow therapeutic window predisposes to toxicity → nausea, diarrhea, CV (↑ HR, arrhythmias) and CNS excitation

- Many drug interactions; toxicity ↑ by erythromycin, cimetidine, and fluoroquinolones
- Aminophylline IV sometimes used in bronchospasm or status asthmaticus

Cromolyn and Nedocromil

- Prevent degranulation of pulmonary mast cells and ↓ release of histamine, PAF, and LTC_4 from inflammatory cells
- Prophylactic use:
  - Decreased symptoms and bronchial hyperactivity (BHR), especially responses to allergens
  - Minimal systemic toxicity but may cause throat irritation and cough
  - Relieved by a β_2 agonist

Glucocorticoids

- Block mediator release and ↓ BHR via ↓ PGs, LTs, and inflammatory interleukins (ILs)
- Surface-active drugs (budesonide, flunisolide) used via inhalation for both acute attacks and for prophylaxis
- May cause oropharyngeal candidiasis (prevented with spacers and gargling)
- Low dosage may also prevent the desensitization of β receptors that can occur with overuse of β_2 agonist
- Prednisone (oral) and IV steroids generally reserved for severe acute attacks

Antileukotrienes

- Zafirlukast and montelukast are antagonists at LTD_4 receptors with slow onset of activity used prophylactically for many forms of asthma, including antigen, exercise, or drug-induced (e.g., ASA).
- Zileuton is a selective inhibitor of lipoxygenases (LOX), ↓ formation of all LTs. It has a more rapid onset (1–3 hours) and is adjunctive to steroids.

Clinical Correlate

All asthmatics need a short-acting beta-2 agonist for acute attacks. For prophylaxis, glucocorticoids are most often used.

Clinical Correlate

For COPD (emphysema, chronic bronchitis), multiple bronchodilators are used including beta-2 agonists and M blockers.

Inflammatory Disorder Drug List and Practice Questions

9

Histamine and Antihistamines

- H_1 antagonists: diphenhydramine, promethazine, meclizine, hydroxyzine, loratadine
- H_2 antagonists: cimetidine, ranitidine, famotidine

Drugs Used in Gastrointestinal Dysfunction

- Proton pump inhibitor: omeprazole and other prazoles
- PGE_1 analog: misoprostol
- Polymer: sucralfate

Drugs Acting on Serotonergic Systems

- $5HT_{1a}$ partial agonist: buspirone
- $5HT_{1d}$ agonist: sumatriptan and other triptans
- $5HT_2$ antagonist: cyproheptadine, atypical antipsychotics
- $5HT_3$ antagonist: ondansetron and other setrons

Antiemetics

- DA antagonist: metoclopramide, prochlorperazine
- H_1 antagonist: meclizine, promethazine
- Muscarinic antagonist: scopolamine
- Cannabinoid: dronabinol
- $5HT_3$ antagonist: ondansetron
- NK_1 antagonist: aprepitant

NSAIDs

- Aspirin, indomethacin, ibuprofen, naproxen, sulindac
- COX 2 inhibitor: celecoxib

Other

- Acetaminophen

Glucocorticoids

- Prednisone, triamcinolone, dexamethasone, hydrocortisone

Drugs Used for Treatment of Gout

- Acute: colchicine, indomethacin
- Chronic: allopurinol, probenecid, febuxostat, pegloticase

Drugs Used for Treatment of RA

- NSAIDs
- DMARDs: methotrexate, etanercept, infliximab, anakinra, and others

Drugs Used for Treatment of Asthma

- β_2 agonists: albuterol, terbutaline
- M-blocker: ipratropium, tiotropium
- Methylxanthine: theophylline
- Mast-cell stabilizer: cromolyn
- Steroids: flunisolide
- LT modifiers: montelukast, zafirlukast, zileuton

PRACTICE QUESTIONS

1. A patient using NSAIDs for chronic pain develops a bleeding ulcer. What drug is designed to selectively treat ulcers of this type?

   A. Famotidine
   B. Bismuth
   C. Aluminum hydroxide
   D. Misoprostol
   E. Muscarinic antagonists

2. Acute poisoning with acetaminophen often requires the use of a specific antidote. The beneficial property of this antidote is that it

   A. supplies sulfhydryl groups to detoxify a reactive metabolite
   B. induces P450 enzymes to enhance elimination
   C. blocks the metabolism of acetaminophen
   D. enhances renal clearance of acetaminophen
   E. chelates acetaminophen

3. Which glucocorticoid is most likely to cause sodium and water retention?

   A. dexamethasone
   B. betamethasone
   C. cortisol
   D. celecoxib
   E. desmopressin

4. A patient with RA is being treated with ibuprofen, but joint pain and stiffness are increasing. His physician prescribes another drug to be used with ibuprofen that may slow progression of the disease. Unfortunately, side effects develop, including dizziness, tinnitus, blurred vision, and pruritus. Ocular examination reveals corneal deposits and slight retinal pigmentation. What is the drug?

   A. Gold salts
   B. Etanercept
   C. Hydroxychloroquine
   D. Methotrexate
   E. Thioridazine

5. A patient suffers from troublesome allergic rhinitis due to pollen, and you want to prescribe a drug for her that is least likely to cause sedation. What would your best choice be?

   A. Betamethasone
   B. Cimetidine
   C. Hydroxyzine
   D. Loratadine
   E. Metoclopramide

6. The widely used anticoagulant warfarin is often implicated in drug interactions. If a patient takes warfarin but later begins self-medicating for ulcer pain, what drug useful for ulcers would increase the risk for bleeding?

   A. Ranitidine
   B. Sucralfate
   C. Misoprostol
   D. Cimetidine
   E. Metoclopramide

7. A patient with a migraine headache is treated with sumatriptan. This drug is beneficial because it

   A. blocks $5HT_3$ receptors
   B. stimulates $5HT_{1D}$ receptors
   C. blocks $5HT_4$ receptors
   D. stimulates $5HT_2$ receptors
   E. blocks muscarinic receptors

8. A child suffering from asthma is to be treated with a drug that blocks the synthesis of leukotrienes. What drug would be an appropriate choice?

   A. Cromolyn
   B. Montelukast
   C. Ipratropium
   D. Zileuton
   E. Theophylline

9. Which one of the following is likely to be used in motion sickness, and nausea and vomiting of pregnancy?

   A. Loratadine
   B. Ondansetron
   C. Meclizine
   D. Fexofenadine
   E. Cimetidine

10. For temporary maintenance of a patent ductus arteriosus prior to cardiac surgery in an infant, what is the drug of choice?

   A. Alprostadil
   B. Indomethacin
   C. Epoprostenol
   D. Celecoxib
   E. Zileuton

11. Following an overdose of an over-the-counter drug, a young college student has marked gastrointestinal distress and is lethargic and confused, with an elevated body temperature. Lab analysis of blood reveals: pCO_2, ↓ HCO_3^-, ↓ K^+, and an anion gap acidosis. The most likely cause of these signs and symptoms is a toxic dose of

   A. acetaminophen
   B. acetylsalicylic acid
   C. diphenhydramine
   D. pseudoephedrine
   E. naproxen

12. Which statement below is accurate regarding aspirin overdose?

   A. N-acetylcysteine should be given immediately
   B. The metabolism rate of aspirin is first-order
   C. Elimination rate is directly proportional to plasma concentration.
   D. Increasing urinary pH would be beneficial
   E. Plasma concentrations decrease exponentially with time.

13. Which one of the following antiinflammatory drugs used in rheumatoid arthritis can bind directly tumor necrosis factor?

A. Etanercept
B. Sulfasalazine
C. Prednisone
D. Celecoxib
E. Penicillamine

14. When used in the management of asthma, glucocorticoids are likely to cause

A. hypoglycemia
B. decreases in blood pressure
C. anabolic actions in wound healing
D. oral thrush
E. sedation

15. A reasonable explanation for the therapeutic effects of ibuprofen or naproxen in primary dysmenorrhea is that these drugs

A. ↓ PGE_2 and $PGF_{2\alpha}$
B. selectively inhibit COX 2
C. ↓ LTB_4
D. inhibit PLA_2
E. PI_2

16. When a patient is started on an appropriate drug for chronic gout it is observed that that the plasma levels of uric acid decrease while the urine levels of uric acid increase. What drug was the patient treated with?

A. Allopurinol
B. Acetylsalicylic acid
C. Indomethacin
D. Colchicine
E. Probenecid

17. The plasma levels of ketoconazole are lower than normal following its oral absorption in patients treated with lansoprazole. What is the reason for this?

A. The induction of enzymes that metabolize ketoconazole
B. Ketoconazole requires an acid environment for its oral absorption
C. Lansoprazole binds acidic drugs in the gastrointestinal tract
D. Increased gastrointestinal transit time because of the prokinetic effects of lansoprazole
E. Competition for transport mechanisms in the gastrointestinal tract

18. Cromolyn useful in many patients with asthma because it

   A. inhibits COX 2
   B. blocks adenosine receptors in bronchiolar smooth muscle
   C. prevents antigen-induced degranulation of mast cells
   D. inhibits phosphodiesterase
   E. ↓ mRNA for IL-2

19. Which one of the following is able to effectively lower intraocular pressure?

   A. Latanoprost
   B. Ergonovine
   C. Atropine
   D. Terbutaline
   E. Morphine

20. Cancer patients being treated with 6-MP may require a dosage adjustment if they are concurrently treated for which of the following?

   A. Constipation
   B. Malaria
   C. Chronic gout
   D. Arthritis
   E. Headache

21. Constipation is highly unlikely to occur with the use of which of the following?

   A. Diphenhydramine
   B. Docusate
   C. Promethazine
   D. Loperamide
   E. Scopolamine

22. A 2-year-old child is brought into the emergency department in convulsions. According to her mother, she had ingested most of a bottle of "sleeping pills," an over-the-counter preparation. What do the sleeping pills she ingested probably contain?

   A. Caffeine
   B. Chlorpromazine
   C. Diphenhydramine
   D. Meperidine
   E. Temazepam

23. In a person who regularly consumes ethanol daily, the potential for hepatotoxicity due to acetaminophen is greater than normal. What is the most likely explanation for this?

    A. Cirrhosis of the liver
    B. Ethanol inhibits the metabolism of acetaminophen
    C. Most beer drinkers are smokers, and nicotine sensitizes the liver to toxins
    D. Nutritional deficiency
    E. Ethanol induces P450 enzymes that form a toxic metabolite

ANSWERS AND EXPLANATIONS

1. **Answer: D.** Misoprostol is a prostaglandin analog indicated for specific use in NSAID-induced ulcers since NSAIDs inhibit the synthesis of protective GI prostaglandins. Other answer choices may be of benefit in this type of ulcer but none are selectively used for NSAIDs.

2. **Answer: A.** Acetaminophen is metabolized primarily by glucuronidation to an inactive metabolite. A minor pathway for metabolism involves cytochrome P450 conversion of acetaminophen to a reactive metabolite that damages the liver. The reactive metabolite is rapidly inactivated normally by glutathione. Prompt administration of N-acetylcysteine is useful because, like glutathione, it supplies sulfhydryl groups to bind the reactive species.

3. **Answer: C.** Various glucocorticoids have different abilities to affect the mineralocorticoid receptor to cause sodium and water retention (an aldosterone-like effect). Generally, the more potent the glucocorticoid, the less likely it is to have an aldosterone effect. Cortisol is a weak glucocorticoid that is equally effective at stimulating mineralocorticoid receptors and thus has sodium and water retention as a property.

4. **Answer: C.** Ocular toxicity is characteristic of chloroquine and hydroxychloroquine. Corneal deposits are reversible, but retinal pigmentation can ultimately lead to blindness. Patients will complain about gastrointestinal distress, visual dysfunction, ringing in the ears (note that tinnitus also occurs in salicylism), and "itchy skin." Hydroxychloroquine also promotes oxidative stress that can lead to hemolysis in G6PD deficiency. DMARDs include gold salts (e.g., auranofin), methotrexate, and etanercept, but thioridazine is a phenothiazine used as an antipsychotic; it lacks an antiinflammatory effect, but does cause retinal pigmentation.

5. **Answer: D.** The usual choice for pollen-induced allergies would be an H_1 antagonist. Of the two listed, loratadine would be the best choice in this case because it does not cross the blood–brain barrier and is nonsedating; hydroxyzine is an effective CNS depressant used for preoperative sedation. Cromolyn (not listed) can also be used in allergic rhinitis and is also nonsedating. Betamethasone, a potent antiinflammatory steroid, is less effective than antihistamines in this situation and would cause more serious side effects. Metoclopramide is a DA-receptor antagonist and prokinetic used as an antiemetic and in GERD. Cimetidine is the prototype H_2 antagonist used in gastrointestinal ulcers.

6. **Answer: D.** Cimetidine is an inhibitor of the hepatic cytochrome P450 isoform that metabolizes warfarin, consequently decreasing its clearance and thus increasing its elimination half-life. The hepatic metabolism of many other drugs can be inhibited by cimetidine, possibly necessitating dose reductions to avoid toxicity, including beta blockers, isoniazid, procainamide, metronidazole, tricyclic antidepressants, and phenytoin.

7. **Answer: B.** It is important to be able to match the serotonin drugs with their respective receptors. The “triptans” used in migraine headaches are agonists at the $5HT_{1D}$ receptor.

8. **Answer: D.** Zileuton blocks the enzyme 5-lipoxygenase which prevents the formation of leukotrienes. This drug is one of many adjuncts available in asthma. Montelukast blocks leukotriene receptors but has no effect on the synthesis of leukotrienes.

9. **Answer: C.** Meclizine is a first-generation antihistamine that effectively penetrates the CNS. Like all first-generation drugs it also blocks muscarinic receptors. Blocking the H_1 and muscarinic receptors are beneficial in motion sickness and nausea and vomiting in pregnancy. Second-generation drugs like loratadine and fexofenadine don’t effectively penetrate the CNS and are of no benefit in these conditions.

10. **Answer: A.** During fetal development, the ductus arteriosus is kept open by prostaglandins. For temporary maintenance of patency in the infant, the PGE_1 analog alprostadil is used. Closure of the ductus in the infant can often be accomplished by intravenous indomethacin, which ↓ PG synthesis by inhibiting COX. Epoprostenol is a prostacyclin analog used in primary pulmonary hypertension.

11. **Answer: B.** If the patient had been able to mention tinnitus, this would be a classic case of aspirin poisoning. At high salicylate blood levels, the combination of effects leading to respiratory depression (respiratory acidosis) and metabolic acidosis results in the observed pH and electrolyte changes, the anion gap (a marker for acidosis), and hyperthermia.

12. **Answer: D.** Back to basic principles. Zero-order elimination means that plasma levels of a drug decrease linearly with time. This occurs with ASA at toxic doses, with phenytoin at high therapeutic doses, and with ethanol at all doses. Enzymes that metabolize ASA are saturated at high plasma levels → constant rate of metabolism = zero-order kinetics. Remember that application of the Henderson-Hasselbalch principle can be important in drug overdose situations. In the case of aspirin, a weak acid, urinary alkalinization favors ionization of the drug →↓ tubular reabsorption → renal elimination. N-acetylcysteine is the antidote for acetaminophen.

13. **Answer: A.** Etanercept binds directly to tumor necrosis factor (TNF), resulting in the inactivation of this cytokine, which plays a major role in a number of inflammatory disorders, including Crohn disease and rheumatoid arthritis. In the synovium, TNF recruits inflammatory cells and leads to angiogenesis and joint destruction. Infliximab, a monoclonal antibody, also inactivates TNF.

14. **Answer: D.** Most often glucocorticoids are used in the treatment of asthma not controlled by a beta-2 agonist inhaler alone. The glucocorticoid is often given by metered dose inhaler when enhances the risk of oral candidiasis (thrush). This can be avoided by rinsing the mouth thoroughly and by using spacers. All of the other effects listed are "opposites," so anticipate possible hyperglycemia, hypertension, decreased wound healing, and CNS excitatory effects that have been interpreted as psychosis.

15. **Answer: A.** PGE_2 and $PGF_{2\alpha}$ both increase in primary dysmenorrhea, and the therapeutic effects of NSAIDs appear to be due to inhibition of the synthesis of these prostaglandins. Both ibuprofen and naproxen are nonselective COX inhibitors that can inhibit the synthesis of prostacyclin (PGI_2). NSAIDs do not inhibit phospholipase A_2, and they do not decrease leukotrienes.

16. **Answer: E.** In chronic gout, the strategy is to decrease uric acid formation from purines by inhibiting xanthine oxidase with allopurinol or increasing urate elimination with uricosurics such as probenecid. Probenecid blocks the tubular reabsorption of uric acid which lowers blood levels of uric acid but results in uricosuria. Colchicine and NSAIDs are less effective and cause more side effects when used in chronic gout. There are preferred In acute gout attacks. Although ASA is uricosuric at antiinflammatory doses, its toxicity makes the drug a poor choice.

17. **Answer: B.** Several drugs, including ketoconazole and fluoroquinolones, require an acidic environment in the gastrointestinal tract for effective absorption into the systemic circulation. Drugs used in treatment of gastrointestinal ulcers such as proton pump inhibitors (lansoprazole) commonly increase gastric pH, leading to the ↓ absorption of such drugs and, consequently, a ↓ in their effects.

18. **Answer: C.** Cromolyn is a mast-cell stabilizer used in asthma (especially antigen-induced) and in food allergies. Inhibition of degranulation with decreased release of histamine and eicosanoids contributes to its antiinflammatory effectiveness in asthma, where it is used for prophylaxis. Methylxanthines, such as theophylline, exert bronchodilating effects via their inhibition of phosphodiesterases and their antagonism of adenosine receptors. Steroids used in asthma ↓ bronchial hyperactivity by several mechanisms, including inhibition of interleukin synthesis. COX 2 inhibitors have no established role in asthma management.

19. **Answer: A.** Latanoprost is a prostaglandin $F_{2\alpha}$ analog that is used in glaucoma to lower intraocular pressure. Ergonovine causes smooth muscle contraction (both uterine and vascular) and is used for control of postpartum hemorrhage. Atropine has the potential to raise intraocular pressure and precipitate glaucoma. Neither a β_2 agonist (terbutaline) nor opioids (morphine) is useful in glaucoma.

20. **Answer: C.** Allopurinol is a uricosuric drug used in chronic gout that prevents formation of uric acid from purines by acting as a suicide substrate of xanthine oxidase. The drug is commonly used in patients undergoing treatment of cancer to slow down formation of uric acid derived from purines released by the cytotoxic action of drugs or radiation. The metabolism of 6-mercaptopurine (6-MP), a substrate for xanthine oxidase, is also inhibited by allopurinol, necessitating a major dose reduction to avoid its toxic effects.

21. **Answer: B.** Docusate is a stool-softening laxative that facilitates mixing of oil and water via its surfactant properties. Drugs that have muscarinic blocking effects, such as scopolamine and the antihistamines diphenhydramine and promethazine, tend to cause constipation by decreasing gastrointestinal motility. Loperamide is an opioid derivative, with no analgesic activity, used in the treatment of diarrheal states.

22. **Answer: C.** Over-the-counter (OTC) sleep aids invariably contain sedating antihistamines such as diphenhydramine. Sometimes called sedative-autonomics, overdoses of such drugs are dangerous, especially in small children. They usually have muscarinic-blocking (atropine-like) effects causing hyperthermia, and they lower the seizure threshold, leading to convulsions. Chlorpromazine is very similar in its pharmacology but is not available OTC and would not be appropriate as a sleeping aid because of its autonomic side effects. Temazepam, a benzodiazepine, is used as a sleeping pill but requires a prescription and raises the seizure threshold. Meperidine is an opioid-analgesic that can cause seizures in OD, but it is not used as a sleeping aid or available OTC. Caffeine is a CNS stimulant.

23. **Answer: E.** Ethanol has mixed effects on liver metabolism of drugs. Acutely, it can act as an enzyme inhibitor, but chronic use may lead to enzyme induction. Acetaminophen is metabolized mainly via conjugation reactions, but a minor pathway involving P-450 (probably the CYP2E1 isoform) results in formation of small amounts of the reactive metabolite, which is (normally) rapidly inactivated by GSH. The chronic ingestion of more than average amounts of ethanol induces the formation of the P-450 isozyme that converts acetaminophen to its reactive metabolite. Thus, more-than-normal amounts of *N*-acetyl-benzoquinoneimine would be formed in an overdose situation, resulting in enhanced hepatotoxicity.

PART VII

Drugs Used in Blood Disorders

Anticoagulants 1

Learning Objectives

- ❑ Compare the use and toxicities of heparin and warfarin

ANTICOAGULANT OVERVIEW

Blood coagulates by transformation of soluble fibrinogen into insoluble fibrin. Circulating proteins interact in a "cascade," where clotting factors undergo limited proteolysis to become active serine proteases. Anticoagulants are drugs which decrease the formation of fibrin clots.

- Oral anticoagulants (e.g., warfarin) inhibit the hepatic synthesis of clotting factors II, VII, IX, and X.
- Heparin inhibits the activity of several activated clotting factors (especially factors IIa and Xa) via its activation of antithrombin III.
- The endogenous anticoagulants, protein C and protein S, cause proteolysis of factors Va and VIIIa.

Clotting Cascade

High-Yield

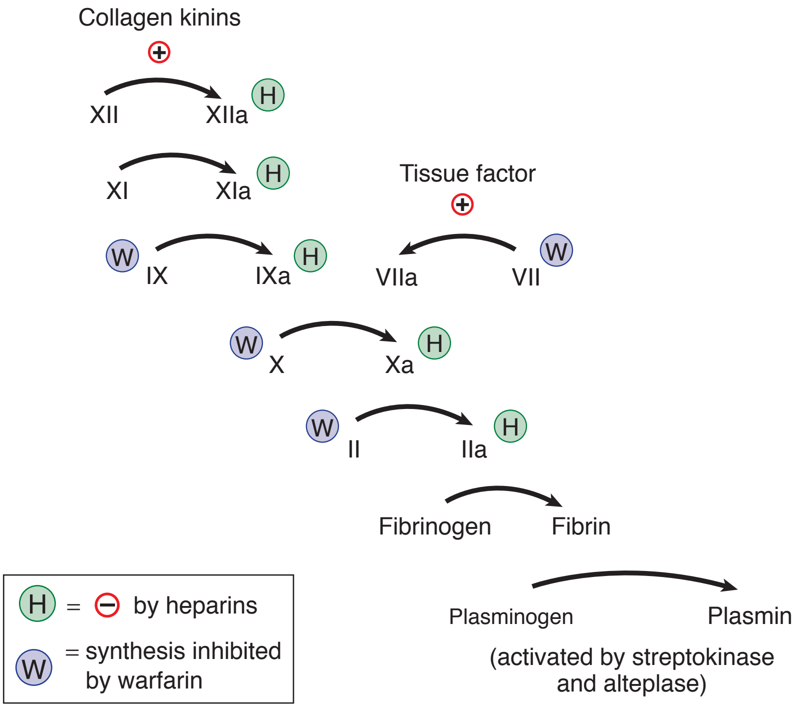


Figure VII-1-1. Actions of Blood Drugs

Comparative Properties of Heparin and Warfarin

High-Yield

Table VII-1-1. Properties of Heparin and Warfarin (Coumarins)

Feature	Heparin(s)	Warfarin (Coumarins)
Chemical nature	Large polysaccharide, water-soluble	Small molecule, lipid-soluble derivatives of vitamin K
Kinetics	Given parenterally (IV, SC), hepatic and reticuloendothelial elimination, half-life = 2 h, no placental access	Given orally, 98% protein bound, PO, liver metabolism, half-life = 30+ h, placental access
Mechanism	Heparin catalyzes the binding of antithrombin III (a serine protease inhibitor) to factors IIa, IXa, Xa, XIa, and XIIa, resulting in their rapid inactivation	↓ Hepatic synthesis of vitamin K–dependent factors II, VII, IX, X—coumarins prevent γ-carboxylation by inhibiting vitamin K epoxide reductase; no effect on factors already present. *In vivo* effects only
Monitoring	Partial thromboplastin time (PTT)	Prothrombin time (PT); INR
Antagonist	Protamine sulfate—chemical antagonism, fast onset	Vitamin K—↑ cofactor synthesis, slow onset; fresh frozen plasma (fast)
Uses	Rapid anticoagulation (intensive) for thromboses, emboli, unstable angina, disseminated intravascular coagulation (DIC), open-heart surgery, etc.	Longer-term anticoagulation (controlled) for thromboses, emboli, post-MI, heart valve damage, atrial arrhythmias, etc.
Toxicity	Bleeding, osteoporosis, heparin-induced thrombocytopenia (HIT), hypersensitivity	Bleeding, skin necrosis (if low protein C), drug interactions, teratogenic (bone dysmorphogenesis)

Heparin

Heparin is a mixture of sulfated polysaccharides with molecular weights of 15–20,000 daltons. Low-molecular-weight (LMW) heparins (e.g., enoxaparin) have a potential advantage of longer half-life, less thrombocytopenia, and possible enhanced activity against factor Xa.

Warfarin

High-Yield

- Drug interactions:
  - Acidic molecule: oral absorption ↓ by cholestyramine
  - Extensive (but weak) plasma protein binding: displacement by other drugs may increase free fraction →↑ PT (e.g., ASA, sulfonamides, phenytoins)
  - Slow hepatic metabolism via P450:
    - Inducers (barbiturates, carbamazepine, rifampin) →↓ PT
    - Inhibitors (cimetidine, macrolides, azole antifungals) →↑ PT
- Protein C deficiency:

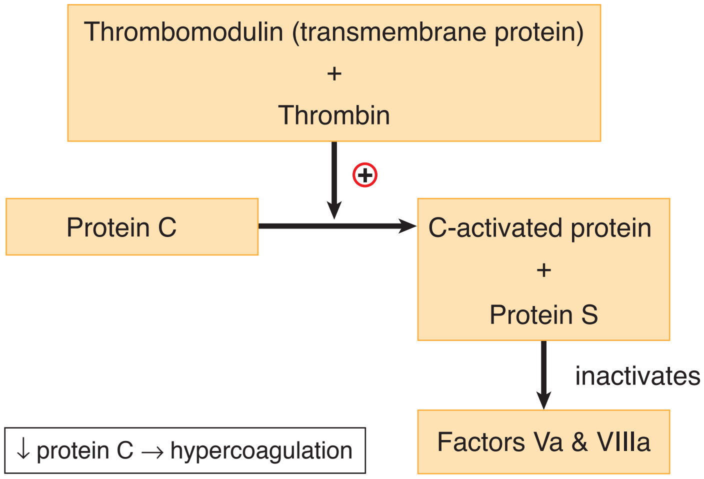


Figure VII-1-2. Activation and Role of Protein C

  - Transient protein C deficiency can be induced when initiating treatment with warfarin because factors VII and protein C have the shortest half-lives of the coagulation factors.

Table VII-1-2. Coagulation Factor Half-Lives

Factor	IIa	VIIa	IXa	Xa	C
Half-life (h)	60	8	24	40	14

  - Consequently, the extrinsic pathway and protein C system are inactivated, whereas the intrinsic system remains active for a few days. Hypercoagulability occurs, which may result in dermal vascular thrombosis and skin necrosis.

Direct Inhibitors of Activated Clotting Factors

Direct thrombin inhibitors

- Directly inhibit thrombin and do not require antithrombin III
- Drugs
  - Argatroban
    - Does not interact with heparin-induced antibodies
    - Used in HIT
  - Dabigatran
    - Oral anticoagulant that does not require monitoring of PT or INR
    - Used in atrial fibrillation as an alternative to warfarin
    - Rapidly reversed by idarucizumab
  - Bivalirudin
    - Used with aspirin in unstable angina when undergoing percutaneous transluminal coronary angioplasty (PTCA)

Direct Factor Xa Inhibitors: Rivaroxaban and Other "-xabans"

- Factor Xa inhibitor, does not require monitoring of PT or INR
- Used to prevent DVTs after knee/hip surgery; prevention of stroke and systemic embolism in non-valvular atrial fibrillation

Thrombolytics 2

Learning Objectives

❑ Describe the clinical features of commonly used fibrinolytic agents

THROMBOLYTIC OVERVIEW

Also called fibrinolytics, thrombolytics lyse thrombi by catalyzing the formation of the endogenous fibrinolytic plasmin (a serine protease) from its precursor, plasminogen. These agents include tissue plasminogen activator (tPA, recombinant) and streptokinase (bacterial). They are used intravenously for short-term emergency management of coronary thromboses in myocardial infarction (MI), deep venous thrombosis, pulmonary embolism, and ischemic stroke (tPA).

Drugs

High-Yield

- Streptokinase
  - Acts on both bound and free plasminogen (not clot specific), depleting circulating plasminogen and factors V and VIII
  - Is antigenic (foreign protein derived from β-hemolytic streptococci); may cause a problem if recent past use or infection—strep antibodies may ↓ activity
- Alteplase (tPA)
  - Clot specific, acting mainly on fibrin-bound plasminogen, the natural activator, so no allergy problems

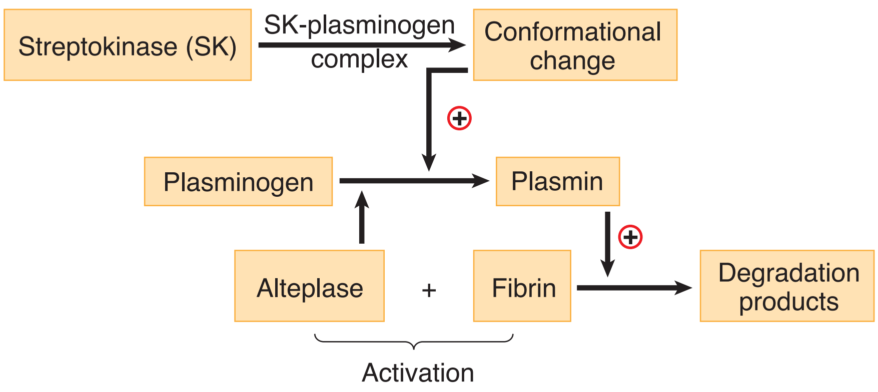


Figure VII-2-1. Actions of Streptokinase and Alteplase

Clinical Features

The overriding factor in effectiveness is early administration, e.g., >60% decrease in mortality post-MI if used within 3 hours.

- ASA, beta blockers, and nitrates further ↓ mortality, and adenosine ↓ infarct size
- Complications include bleeding, possible intracerebral hemorrhage
- Streptokinase may cause hypersensitivity reactions and hypotension
- Antifibrinolysins (aminocaproic and tranexamic acids)—possible antidotes in excessive bleeding

Antiplatelet Drugs 3

Learning Objectives

❑ List the commonly used antiplatelet agents and their distinguishing features

ANTIPLATELET OVERVIEW

Thrombus (clot) formation involves:

- Platelet adhesion to site of vascular injury
- Activation of platelets by factors that include TxA_2, ADP, collagen, 5HT, and thrombin →↑ expression of glycoprotein IIb/IIIa receptors
- Aggregation of platelets by a cross-linking reaction due to fibrinogen binding to glycoprotein IIb/IIIa receptors

Platelet Activation

High-Yield

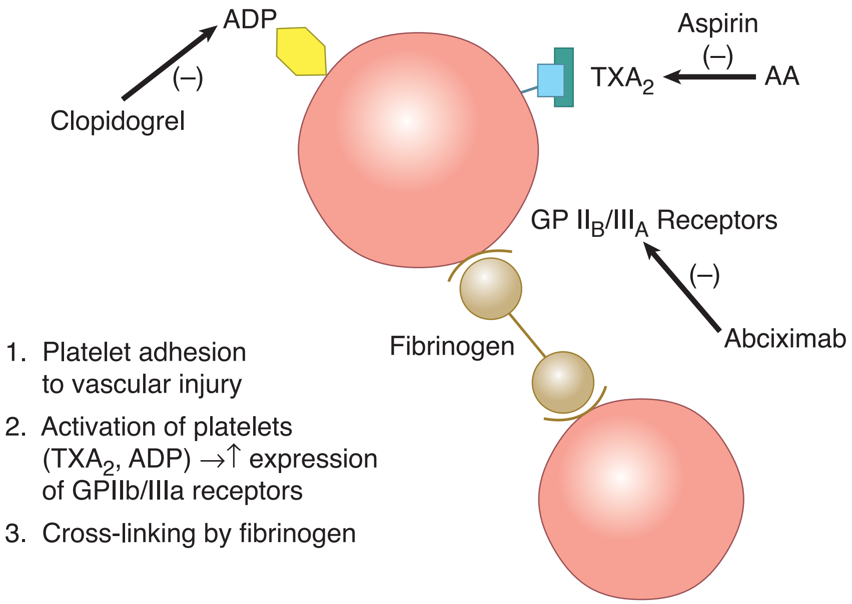


Figure VII-3-1. Platelet Activation

Note

Platelet Aggregation

Increased by ADP, 5HT, TxA_2, thrombin, $\alpha_2$agonists

Decreased by PGI_2, cAMP, ASA, clopidogrel, GP IIb/IIIa blockers

Drugs

High-Yield

- Aspirin
  - Irreversibly inhibits COX in platelets →↓ activation
  - Low doses prevent MI and recurrence; prophylaxis in atrial arrhythmias and TIAs
  - Adverse effects (*see* Section VI, Drugs for Inflammatory and Related Disorders)
- Clopidogrel, prasugrel, ticagrelor
  - Block ADP receptors on platelets →↓ activation
  - Alternatives to ASA in TIAs, post-MI, and unstable angina
  - Aspirin + ADP receptor blockers are used in patients with non-ST elevation ACS
  - Hemorrhage, leukopenia, and thrombocytopenic purpura
- Abciximab, eptifibatide, and tirofiban
  - Antagonists that bind to glycoprotein IIb/IIIa receptors →↓ aggregation by preventing the cross-linking reaction
  - Used mainly in acute coronary syndromes and postangioplasty

Blood Disorder Drug List and Practice Questions

4

Anticoagulants

- Heparin
- Warfarin
- Argatroban
- Bivalirudin
- Dabigatran
- Rivaroxaban

Thrombolytics

- Alteplase (tPA)
- Streptokinase

Antiplatelet

- Aspirin
- Clopidogrel
- Abciximab
- Prasugrel
- Ticagrelor

PRACTICE QUESTIONS

1. Which of the following compounds is most likely to block ADP receptors and prevent platelet aggregation?

   A. Clopidogrel
   B. Aspirin
   C. Prostacyclin
   D. Abciximab
   E. Montelukast

2. A woman who has a mechanical heart valve and who is taking warfarin informs you that she hopes to get pregnant in the near future. What advice should she receive regarding her antithrombotic medication during the anticipated pregnancy?

   A. Warfarin should be continued until the third trimester.
   B. Warfarin should be replaced with aspirin at analgesic doses.
   C. All medications that affect the blood should be discontinued.
   D. Warfarin should be replaced with heparin.
   E. Warfarin should be discontinued, and supplementary vitamin K taken throughout the pregnancy.

3. The primary advantage of enoxaparin over heparin is that it

   A. is unlikely to cause bleeding
   B. more effectively Inhibits the synthesis of clotting factors
   C. has a more rapid onset
   D. does not case thrombocytopenia
   E. has a longer half-life

4. Which of the following statements regarding warfarin is true?

   A. It is a prodrug converted to its active metabolite spontaneously in the blood.
   B. It has low lipophilicity and does not cross the placental barrier.
   C. It causes a depletion in protein C before it decreases prothrombin.
   D. It inhibits release of vitamin K–dependent clotting factors from hepatocytes.
   E. It is inactivated by protamine.

5. Which of the following statements is true regarding the parenteral administration of alteplase?

   A. It increases the formation of plasminogen.
   B. It is less effective than streptokinase when given after a myocardial infarction.
   C. It causes a high incidence of thrombocytopenia.
   D. It may cause bleeding reversible by aminocaproic acid.
   E. It activates free plasminogen.

6. Following a myocardial infarction, a patient is stabilized on warfarin, the dose being adjusted to give a prothrombin time of 22 seconds. Which of the following statements regarding potential drug interactions in this patient is accurate?

   A. Cholestyramine will increase prothrombin time.
   B. Cimetidine is likely to decrease prothrombin time.
   C. Antibacterial sulfonamides may enhance the effects of warfarin.
   D. Vitamin K would restore prothrombin time to normal within 30 minutes.
   E. If this patient takes half an aspirin tablet daily, the dose of warfarin will need to be increased.

ANSWERS AND EXPLANATIONS

1. **Answer: A.** Platelet aggregation is stimulated by many compounds, including ADP, thromboxane A_2, fibrin, and serotonin. Clopidogrel, along with ticlopidine, blocks ADP receptors and prevent platelet activation. Prostacyclin (PGI_2) from endothelial cells is a naturally occurring compound that inhibits platelet aggregation by stimulating PGI_2 receptors. Aspiring inhibits the synthesis of thromboxane A_2. Abciximab is a monoclonal antibody targeted to the glycoprotein IIb/IIIa receptor which inhibits aggregation. Montelukast blocks leukotriene receptors and is used in asthma.

2. **Answer: D.** Discontinuing warfarin is appropriate during pregnancy because it is a known teratogen that causes bone dysmorphogenesis. The patient will need continued protection against thrombus formation, and heparin (or a related low molecular weight compound) is usually advised, despite the fact that the drug will require parenteral administration and can cause thrombocytopenia.

3. **Answer: E.** Enoxaparin is a low-molecular weight heparin. As such, it is smaller and will have a longer half-life compared to heparin. It still has a risk of causing bleeding and thrombocytopenia, but is not more rapid in onset. Heparins do not affect the synthesis of clotting factors, but rather rapidly inactivate existing factors.

4. **Answer: C.** Warfarin inhibits the hepatic synthesis of factors II (prothrombin), VII, IX, and X. Its onset of anticoagulation activity is slow, and its impact on individual coagulation factors depends on their half-lives. Factor VII and protein C have much shorter half-lives than prothrombin, and so the extrinsic pathway and protein C system are the first to be affected by warfarin. The intrinsic pathway continues to function for 2 to 3 days, causing a state of hypercoagulability and possible vascular thrombosis.

5. **Answer: D.** Alteplase is thrombolytic (or “fibrinolytic”) because it activates plasminogen, resulting in the increased formation of plasmin. Its efficacy is equivalent to that of streptokinase, but alteplase has the advantage of only activating plasminogen bound to fibrin (clot specific) but not free plasminogen. All thrombolytics can cause bleeding, which may be counteracted to some extent by administration of antifibrinolysins, such as aminocaproic acid.

6. **Answer: C.** Warfarin binds extensively (98%) but weakly to plasma proteins and can be displaced by other drugs (e.g., ASA, chloral hydrate, phenytoin, sulfinpyrazone, and sulfonamides), resulting in an increase in its anticoagulant effects. Bile acid sequestrants bind acidic drugs such as warfarin, preventing their gastrointestinal absorption (↓ prothrombin time [PT]), and cimetidine, which inhibits the metabolism of warfarin, causing an increase in PT. Vitamin K restores levels of prothrombin and several other coagulation factors, but the action is slow (24 to 48 hours). Due to antiplatelet effects, even low doses of ASA may enhance bleeding in patients on warfarin.

PART VIII

Endocrine Pharmacology

Drugs Used in Diabetes 1

Learning Objectives

- ❏ Use knowledge of insulins to select appropriate dosage forms in clinical situations
- ❏ Describe the mechanism of action and side effects of sulfonylureas, metformin, acarbose, pioglitazone and rosiglitazone
- ❏ Answer questions about agents affecting glucagon-like peptide-1
- ❏ Demonstrate understanding of sodium-glucose cotransporter-2 inhibitor

DIABETES MELLITUS

Type 1 (IDDM)

- Early onset
- Loss of pancreatic B cells → absolute dependence on insulin (diet + insulin ± oral agents)
- Ketoacidosis-prone

Type 2 (NIDDM)

- Usually adult onset
- Decreased response to insulin → (diet → oral hypoglycemics ± insulin)
- Not ketoacidosis-prone

Note

Insulin Release

- Increased by glucose, sulfonylureas, M-agonists, β_2-agonists
- Decreased by α_2-agonists

INSULIN FORMS

Table VIII-1-1. Kinetics (in Hours) of Insulin Forms with Subcutaneous Injection

Form	Onset	Peak Effect	Duration
Lispro*	0.3–0.5	1–2	3–4
Regular*	0.5–1	2–4	5–7
Glargine	1	no peak	≥24

* Only forms that can be used intravenously; peak action in 2 to 4 min.

Clinical Correlate

Diabetic Ketoacidosis

- Symptoms: polyuria, polydipsia, nausea, fatigue, dehydration, Kussmaul breathing, “fruity” breath
- Treatment: regular insulin IV, fluid and electrolyte replacement

- Glargine:
  - Insulin analog with no peak ("peakless," i.e., broad plasma concentration plateau)
  - Ultralong duration of action
  - Used to supply a constant background level
- Mechanism: insulin binds to transmembrane receptors which activate tyrosine kinase to phosphorylate tissue-specific substrates

DRUGS FOR TYPE II DIABETES

Sulfonylureas

High-Yield

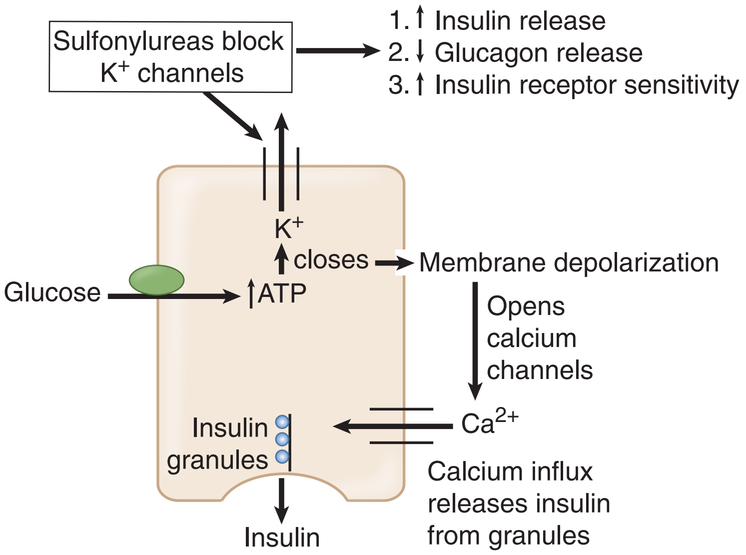


Figure VIII-1-1. Mode of Action of Sulfonylureas

- Mechanisms:
  - Normally, K^+ efflux in pancreatic β cells maintains hyperpolarization of membranes, and insulin is released only when depolarization occurs.
  - Glucose acts as an insulinogen by increasing intracellular ATP → closure of K^+ channels → membrane depolarization →↑ Ca^{2+} influx → insulin release.
  - The acute action of sulfonylureas is to block K^+ channels → depolarization → insulin release.
- Effects of increased insulin:
  - →↓ glucagon release from pancreatic α cells
  - Continued use of sulfonylureas ↑ tissue responses to insulin (especially muscle and liver) via changes in receptor function

Note

Hypoglycemic Reactions

- Symptoms: lip/tongue tingling, lethargy, confusion, sweats, tremors, tachycardia, coma, seizures
- Treatment: oral glucose, IV dextrose if unconscious, or glucagon (IM or inhalation)

Note

Repaglinide

- Mechanisms: stimulates insulin release from pancreatic beta cells
- Use: adjunctive use in type 2 diabetes—administer just before meals due to short half-life

- Drugs:
  - Second generation:
    - Glipizide (↓ dose in hepatic dysfunction)
    - Glyburide (active metabolite, ↓ dose in renal dysfunction)
- Side effects:
  - Hypoglycemia
  - Weight gain
  - Drug interactions mainly with first-generation drugs →↑ hypoglycemia with cimetidine, insulin, salicylates, sulfonamides

Metformin

High-Yield

- "Euglycemic," ↓ postprandial glucose levels, but does not cause hypoglycemia or weight gain
- Mechanisms: may involve ↑ tissue sensitivity to insulin and/or ↓ hepatic gluconeogenesis
- Use: monotherapy or combinations (synergistic with sulfonylureas)
- Side effects: possible lactic acidosis; gastrointestinal distress is common

Acarbose

- No hypoglycemia
- Mechanisms: inhibits α-glucosidase in brush borders of small intestine →↓ formation of absorbable carbohydrate →↓ postprandial glucose →↓ demand for insulin
- Side effects: gastrointestinal discomfort, flatulence, diarrhea; recent concern over potential hepatotoxicity

Thiazolidinediones: Pioglitazone and Rosiglitazone

High-Yield

- Mechanisms: bind to nuclear peroxisome proliferator-activating receptors (PPARγ) involved in transcription of insulin-responsive genes → sensitization of tissues to insulin, plus ↓ hepatic gluconeogenesis and triglycerides and ↑ insulin receptor numbers
- Side effects: less hypoglycemia than sulfonylureas, but weight gain and edema reported

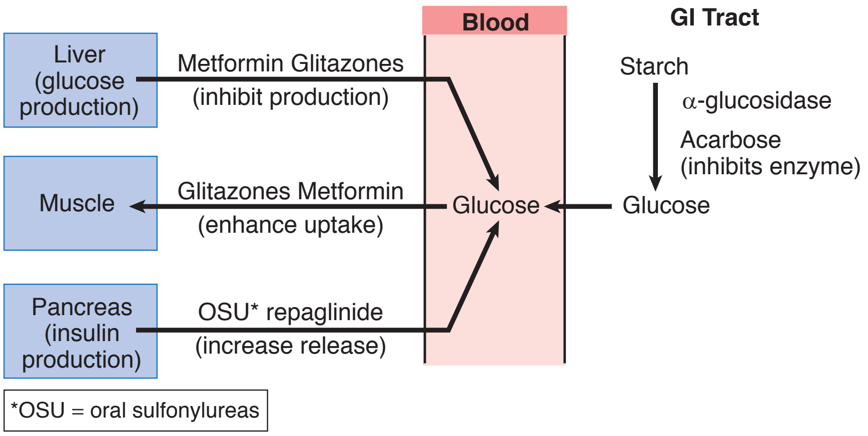


Figure VIII-1-2. Modes of Action of Drugs Used to Treat Diabetes

Recall Question

Which of the following is a side effect of sulfonylureas?

A. Weight loss

B. Bradycardia

C. Hyperglycemia

D. Confusion

Answer: D

Agents Affecting Glucagon-Like Peptide-1 (GLP-1)

Exenatide

- Mechanism: GLP-1 is an incretin released from the small intestine. It augments glucose-dependent insulin secretion. Exenatide is a long-acting GLP-1 receptor full agonist used in combination with other agents in type 2 diabetes.
- Side effects: nausea, hypoglycemia when used with oral sulfonylureas

Sitagliptin and Other Gliptins

- Mechanism: inhibits dipeptidyl peptidase (DPP-4) thereby inhibiting the inactivation of GLP-1

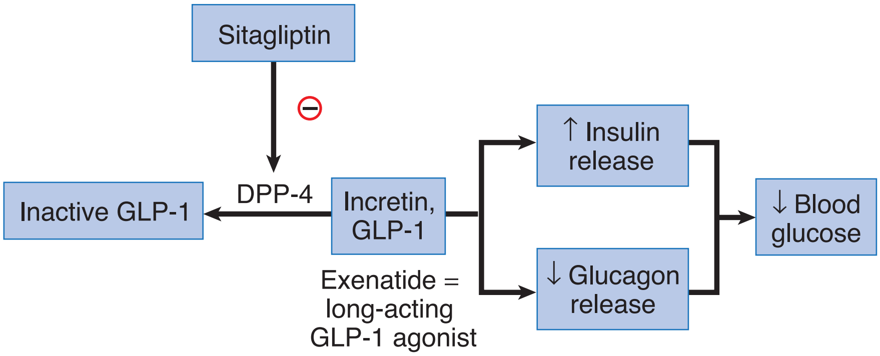


Figure VIII-1-3. Action of Drugs Affecting GLP-1

Sodium-Glucose Cotransporter-2 (SGLT-2) Inhibitor: Canagliflozin and other gliflozins

- Blocks SGLT-2 in the proximal tubule, increasing glucose excretion

Note

Pramlintide is a synthetic version of amylin that slows the rate at which food is absorbed from the intestine, decreases glucose production, and decreases appetite. It is used in type 1 and type 2 diabetes.

Steroid Hormones 2

Learning Objectives

- ❑ Describe clinical situations requiring the use of adrenal steroids, estrogens, and progestin
- ❑ Solve problems concerning oral contraceptives
- ❑ List the common complications of steroid hormone use

ADRENAL STEROIDS

Uses

- Nonendocrine uses: inflammatory disorders (and accompanying adverse effects), *see* Section VI, Drugs for Inflammatory and Related Disorders.
- Endocrine uses of glucocorticoids (e.g., prednisone, dexamethasone, hydrocortisone) and the mineralocorticoid (fludrocortisone) include:
  - Addison disease: replacement therapy
  - Adrenal insufficiency states (infection, shock, trauma): supplementation
  - Premature delivery to prevent respiratory distress syndrome: supplementation
  - Adrenal hyperplasia: feedback inhibition of ACTH

Antagonists

High-Yield

- Adrenal steroid antagonists:
  - Spironolactone
  - Blocks aldosterone and androgen receptors (*see* Section III, Cardiac and Renal Pharmacology)
- Mifepristone: blocks glucocorticoid and progestin receptors
- Synthesis inhibitors: metyrapone (blocks 11-hydroxylation); ketoconazole

ESTROGENS

Estradiol is the major natural estrogen. The rationale for synthetics is to increase oral bioavailability, increase half-life, and increase feedback inhibition of FSH and LH.

Primary drugs include conjugated equine estrogens (Premarin) and ethinyl estradiol and mestranol.

Clinical uses for estrogens include female hypogonadism, hormone replacement therapy (HRT) in menopause leading to decreased bone resorption (decreased PTH), contraception, dysmenorrhea, uterine bleeding, and acne.

Side effects include nausea, breast tenderness, endometrial hyperplasia, increased gallbladder disease, cholestasis, migraine, and bloating. There is an increased risk of blood coagulation via decreased antithrombin III and increased factors II, VII, IX, and X (only at high dose).

There is an increased risk of endometrial cancer with the use of estrogens (unless progestins are added). The increased risk of breast cancer is controversial, but use caution if other risk factors are present.

Estrogen Antagonists

High-Yield

- Anastrozole
  - Mode of action: aromatase inhibitor →↓ estrogen synthesis
  - Use: estrogen-dependent, postmenopausal breast cancer

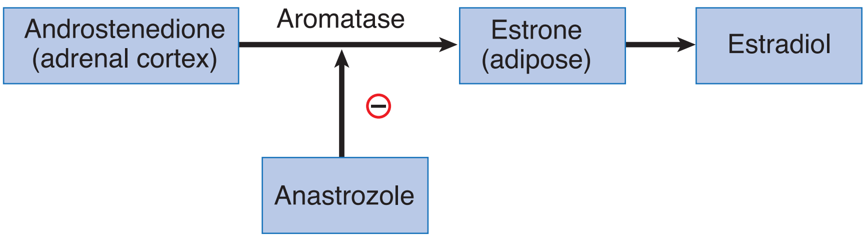


Figure VIII-2-1. Mechanism of Action of Anastrozole

- **Clomiphene** (fertility pill)
  - Mode of action: ↓ feedback inhibition →↑ FSH and LH →↑ ovulation → pregnancy
  - Use: fertility drug
  - Adverse effect: ↑ multiple births

Selective Estrogen-Receptor Modulators (SERMs)

High-Yield

- **Tamoxifen**
  - Variable actions depending on "target" tissue
  - E-receptor agonist (bone), antagonist (breast), and partial agonist (endometrium)
  - Possible ↑ risk of endometrial cancer
  - Used in estrogen-dependent breast cancer and for prophylaxis in high-risk patients
- Raloxifene
  - E-receptor agonist (bone), antagonist breast and uterus
  - When used in menopause, there is no increased cancer risk
  - Use: prophylaxis of postmenopausal osteoporosis, breast cancer

Table VIII-2-1. Comparison of Tamoxifen and Raloxifene in Various Tissues

Drug	Bone	Breast	Endometrium
Tamoxifen	Agonist	Antagonist	Agonist
Raloxifene	Agonist	Antagonist	Antagonist

PROGESTINS

Progesterone is the major natural progestin. The rationale for synthetics is to increase oral bioavailability and increase feedback inhibition of gonadotropins, especially luteinizing hormone (LH).

Primary drugs include medroxyprogesterone, norethindrone, and desogestrel (a synthetic progestin devoid of androgenic and antiestrogenic activities common to other derivatives).

Clinical uses for progestins include contraception (oral with estrogens or depot contraception with medroxyprogesterone IM every 3 months) and HRT (combine with estrogens to decrease endometrial cancer risk).

Side effects include increased HDL and LDL (antiestrogenic), glucose intolerance, breakthrough bleeding, androgenic (hirsutism and acne), weight gain, and depression.

The progestin antagonist mifepristone is used as an abortifacient (with prostaglandins [PGs]).

ORAL CONTRACEPTIVES

Oral contraceptives are combinations of estrogens (ethinyl estradiol, mestranol) with progestins (norgestrel, norethindrone) in varied doses, with mono-, bi-, and triphasic variants. Oral contraceptives suppress gonadotropins, especially midcycle LH surge.

- Side effects: those of estrogens and progestins, as seen previously
- Interactions: ↓ contraceptive effectiveness when used with antimicrobials and enzyme inducers
- Benefits:
  - ↓ risk of endometrial and ovarian cancer
  - ↓ dysmenorrhea
  - ↓ endometriosis
  - ↓ pelvic inflammatory disease (PID)
  - ↓ osteoporosis

ANDROGENS

Androgens include methyltestosterone and 17-alkyl derivatives with increased anabolic actions, e.g., oxandrolone, nandrolone.

- Uses:
  - Male hypogonadism and for anabolic actions →↑ muscle mass, ↑ RBCs, ↓ nitrogen excretion
  - Illicit use in athletics
- Side effects: excessive masculinization, premature closure of epiphysis, cholestatic jaundice, aggression, dependence

Antagonists

High-Yield

- **Flutamide:** androgen receptor blocker: used for androgen-receptor–positive prostate cancer
- **Leuprolide:** GnRH analog: repository form used for androgen-receptor–positive prostate cancer
- **Finasteride**
  - 5-alpha reductase inhibitor, preventing conversion of testosterone to dihydrotestosterone (DHT)
  - DHT is responsible for hair loss and prostate enlargement
  - Uses: BPH, male pattern baldness
  - Caution: teratogenicity

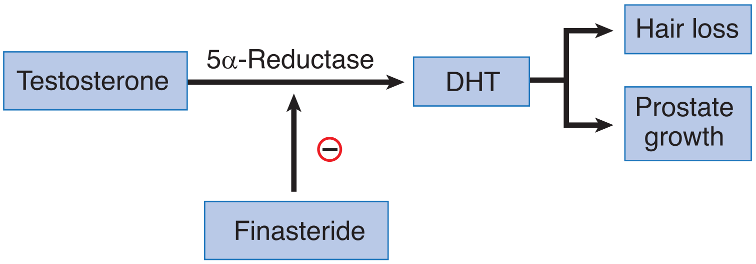


Figure VIII-2-2. Mechanism of Action of Finasteride

Recall Question

Which of the following medications is given to increase the chance of a woman getting pregnant?

A. Mifepristone

B. Clomiphene

C. Tamoxifen

D. Anastrozole

Answer: B

Antithyroid Agents

3

Learning Objectives

- ❏ Describe the short-term effect of iodine on the thyroid and the most commonly used thioamides

THYROID HORMONES

Synthesis and Release

High-Yield

Table VIII-3-1. Synthesis of Thyroid Hormone and Effects of Antithyroid Agents

Thyroid Hormone Synthesis	Effects of Antithyroid Agents
1. Active accumulation of iodide into gland	Basis for selective cell destruction by ^{131}I
2. Oxidation of iodide to iodine by peroxidase	Inhibited by thioamides
3. Iodination of tyrosyl residues (organification) on thyroglobulin to form MIT and DIT	Inhibited by thioamides
4. Coupling of MIT and DIT to form T_3 and T_4	Inhibited by thioamides
5. Proteolytic release of T_3 and T_4 from thyroglobulin	Inhibited by high doses of iodide*
6. Conversion of T_4 to T_3 via 5´ deiodinase in peripheral tissues	Inhibited by propranolol* and propylthiouracil*

MIT, monoiodotyrosine; DIT, diiodotyrosine; T_3, triiodothyronine; T_4, thyroxine

*Thyroid storm management may include the use of any or all of these agents.

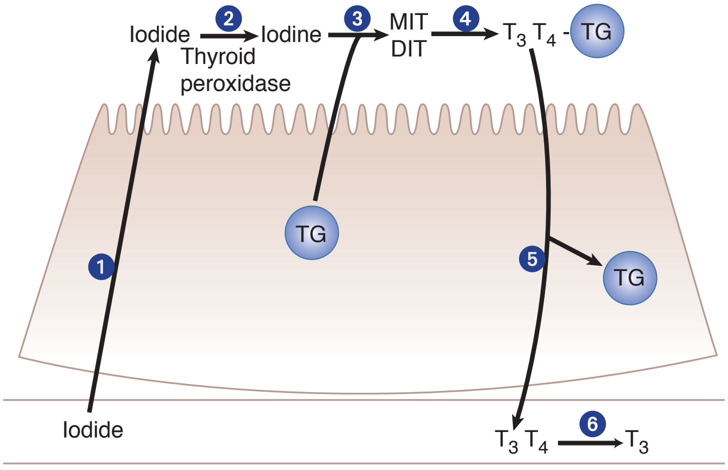


Figure VIII-3-1. Thyroid Hormone Synthesis

Drugs for Hyperthyroidism

- Thioamides: propylthiouracil and methimazole
  - Use: uncomplicated hyperthyroid conditions; slow in onset
  - High-dose propylthiouracil inhibits 5′ deiodinase
  - Common maculopapular rash
  - Both drugs cross the placental barrier, but propylthiouracil is safer in pregnancy because it is extensively protein bound
- Iodide
  - Potassium iodide plus iodine (Lugol’s solution) possible use in thyrotoxicosis: used preoperatively →↓ gland size, fragility, and vascularity
  - No long-term use because thyroid gland “escapes” from effects after 10 to 14 days
- I^{131}: most commonly used drug for hyperthyroidism

Drugs Related to Hypothalamic and Pituitary Hormones

4

Learning Objectives

- ❏ List commonly used pharmacologic agents that directly affect hypothalamic and pituitary hormone release

Table VIII-4-1. Drugs Related to Hypothalamic and Pituitary Hormones

Hormone	Pharmacologic Agent	Clinical Uses
GH	Somatrem or somatropin	Pituitary dwarfism, osteoporosis
Somatostatin	Octreotide	Acromegaly, carcinoid and secretory-GI tumors
ACTH	Cosyntropin	Infantile spasms
GnRH	Leuprolide, nafarelin	Endometriosis, prostate carcinoma (repository form)
FSH and LH	Urofollitropin (FSH), placental HCG (LH), menotropins (FSH and LH)	Hypogonadal states
PIH (DA)	Cabergoline	Hyperprolactinemia
Oxytocin	Oxytocin	Labor induction
Vasopressin	Desmopressin (V2 selective)	• Neurogenic (pituitary) diabetes insipidus • Hemophilia A (↑ factor VIII from liver) • von Willebrand disease (↑ vW factor from endothelium) • Primary nocturnal enuresis

ACTH, adrenocorticotropin hormone; DA, dopamine; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; PIH, prolactin-inhibiting hormone

Clinical Correlate

Drugs useful in the syndrome of inappropriate secretion of ADH (SIADH) include demeclocycline and tolvaptan, which block V2 receptors in the collecting duct. Loop diuretics, salt tablets, and fluid restriction are also useful.

Drugs Used for Bone and Mineral Disorders

5

Learning Objectives

❑ Use knowledge of bisphosphonates and teriparatide to solve problems

OSTEOPOROSIS TREATMENT

Bisphosphonates: Alendronate and other dronates

High-Yield

- Mechanisms: stabilize hydroxyapatite bone structure and also induce osteoblasts to secrete inhibitors of osteoclasts →↓ bone resorption →↓ progression of osteoporosis
- Clinical uses:
  - Established use in Paget disease
  - Efficacy in postmenopausal osteoporosis depends on individual drug, but alendronate is effective and with HRT will increase bone mineral density (BMD)
  - Alendronate is drug of choice for glucocorticoid-induced osteoporosis
- Side effects: bone mineralization defects (etidronate and pamidronate); gastrointestinal distress including esophageal ulcers (alendronate)

Teriparatide

- Mechanism: recombinant DNA PTH analog
- Clinical use: 1x daily to stimulate osteoblasts and new bone formation
- Continuous infusion would stimulate osteoclast activity
- Used for <2 years; may ↑ risk of osteosarcoma

Endocrine Drug List and Practice Questions

6

Table VIII-6-1. Endocrine Drug List

Drugs Used in Diabetes	Antithyroid Drugs
Insulins, glargine	Propylthiouracil
Sulfonylureas—glipizide, glyburide	Methimazole
Metformin	KI and I (Lugol's)
Acarbose	I^{131}
Thiazolidinediones—pioglitazone, rosiglitazone	
GLP-1 drugs—exenatide, sitagliptin	
Steroid Hormones	**Hypothalamic/Pituitary Drugs**
Adrenosteroids	Somatropin
Cortisol	Octreotide
Triamcinolone	Leuprolide
Fludrocortisone	Oxytocin, vasopressin
Prednisone	**Drugs Used in Bone and Mineral Disorders**
Dexamethasone	Alendronate
Hydrocortisone	Teriparatide
Estrogens	
Ethinyl estradiol	
Mestranol	
Tamoxifen (SERM)	
Raloxifene (SERM)	
Progestins	
Medroxyprogesterone	
Norgestrel	
Norethindrone	
Desogestrel	
Mifepristone (antagonist)	
Androgens	
Methyltestosterone	
Oxandrolone	
Flutamide (antagonist)	
Finasteride (5-α-reductase inhibitor)	

PRACTICE QUESTIONS

1. A 70-year-old man is diagnosed with benign prostatic hyperplasia (BPH), and his physician is considering drug treatment of the condition. It was decided that the drug finasteride will be used. The effects of finasteride will result in a decrease in the synthesis of what substance?

    A. Epinephrine
    B. Norepinephrine
    C. Dihydrotestosterone
    D. Testosterone
    E. GnRH

2. Which of the following statements is accurate regarding drug management of hyperthyroidism?

    A. The actions of thyroid peroxidase are inhibited by I^{131}
    B. Propylthiouracil inhibits the conversion of thyroxine to triiodothyronine
    C. Methimazole is unable to cross the placental barrier
    D. Iodide salts can be used for long-term management
    E. The iodination of tyrosyl residues to form MIT and DIT are inhibited by beta blockers

3. What drug is useful to distinguish neurogenic from nephrogenic diabetes insipidus?

    A. Amiloride
    B. Demeclocycline
    C. Desmopressin
    D. Hydrochlorothiazide
    E. Lithium

4. The release of insulin from pancreatic beta cells would most likely be stimulated by which of the following?

    A. Clonidine
    B. Norepinephrine
    C. Diazoxide
    D. Glipizide
    E. Hypoglycemia

5. When used at higher doses than commonly employed for other purposes, what drug can effectively inhibit steroidogenesis in a variety of tissues?

   A. Flutamide
   B. Misoprostol
   C. Clomiphene
   D. Tamoxifen
   E. Ketoconazole

6. In a patient with type 2 diabetes, which drug mimics the action of incretins to augment glucose-dependent insulin secretion?

   A. Acarbose
   B. Glucagon
   C. Exenatide
   D. Metformin
   E. Rosiglitazone

7. To supplement other oral type 2 diabetes medication, a patient is prescribed a drug to inhibit the intestinal absorption of carbohydrates. What would be an appropriate drug?

   A. Metformin
   B. Acarbose
   C. Repaglinide
   D. Insulin lispro
   E. Pioglitazone

8. What is the drug of choice for management of adrenal glucocorticoid-induced osteoporosis?

   A. Alendronate
   B. Calcitonin
   C. Estrogen
   D. Ketoconazole
   E. Vitamin D

9. Which drug has utility in inhibiting the severe secretory diarrhea of hormone-secreting tumors of the pancreas and GI tract, as well as in the treatment of acromegaly?

   A. Octreotide
   B. Leuprolide
   C. Bromocriptine
   D. Sertraline
   E. Anastrozole

ANSWERS AND EXPLANATIONS

1. **Answer: C.** The drug finasteride inhibits the enzyme 5-α reductase, the enzyme that converts testosterone to dihydrotestosterone (DHT). DHT is responsible for prostate enlargement in BPH. The other commonly used drugs in BPH are alpha-1 antagonists such as prazosin and tamsulosin.

2. **Answer: B.** Thioamides used at conventional doses in Graves disease are slow to act; they inhibit iodination and the coupling reactions in hormone synthesis and do not affect the release of stored thyroxine. At high doses, propylthiouracil may act more rapidly because of its inhibition of 5′-deiodinase, preventing the conversion of T_4 to T_3. Thioamides are not teratogenic, and they do not decrease glandular size or vascularity; KI plus iodine (Lugol's solution) is used preoperatively to this end. Use of iodide in hyperthyroidism is only temporary because the thyroid gland "escapes" from its actions within a week or two.

3. **Answer: C.** Neurogenic diabetes insipidus is treated with desmopressin, a drug that is similar to vasopressin (ADH), yet is a selective activator of V_2 receptors in the kidney. Remember that V_1 receptors are present in smooth muscle, and their activation leads to vasoconstriction and bronchoconstriction. Nephrogenic diabetes insipidus (decreased response of vasopressin receptors) is treated with thiazides, except in the case of that induced by lithium, when amiloride is preferred (because thiazides increase blood levels of lithium). In cases where it is necessary to distinguish between neurogenic and nephrogenic, desmopressin is used. Desmopressin would alleviate the symptoms of neurogenic but have no effect in nephrogenic diabetes insipidus.

4. **Answer: D.** The release of insulin from the pancreas is stimulated by insulinogens (glucose), sulfonylurea hypoglycemics (glipizide), activators of beta-2 adrenoceptors (e.g., albuterol), and activators of muscarinic receptors (e.g., pilocarpine). Activation of alpha-2 receptors inhibits insulin release (clonidine and norepinephrine). Hypokalemia, and diazoxide, keep potassium channels on beta cells open resulting in decreased insulin release

5. **Answer: E.** Ketoconazole is an antifungal drug that decreases the synthesis of various steroids including cortisol and testosterone by inhibiting cytochrome P450 enzymes. Flutamide is an androgen-receptor antagonist, and tamoxifen is a partial agonist (or mixed agonist-antagonist) at estrogen receptors. Misoprostol is a prostaglandin analog used in NSAID-induced ulcers. Clomiphene is used to induce ovulation.

6. **Answer: C.** Exenatide is a glucagon-like peptide-1 (GLP-1) analog. GLP-1 is an incretin released from the small intestine that enhances glucose-dependent insulin secretion. Metformin is "euglycemic," lowering elevated glucose levels to the normal range, and acarbose simply prevents postprandial hyperglycemia. Glucagon causes hyperglycemia, an effect that is sometimes employed in management of hypoglycemia. Rosiglitazone increases the sensitivity to insulin by increasing insulin receptor numbers.

7. **Answer: B.** Acarbose inhibits the enzyme α-glucosidase in the brush borders of the small intestine. This decreases the formation of absorbable carbohydrate and thereby decreases glucose absorption. The net effect is that glucose levels after a meal don't rise as significantly and therefore the insulin demand is reduced.

8. **Answer: A.** Alendronate is currently the drug of choice to prevent osteoporosis in patients who must be maintained on steroids for their antiinflammatory and immunosuppressive effects. The drug also decreases bone resorption during menopause and is sometimes favored in patients who are at risk for neoplasias if treated with sex hormones. Care must be taken with alendronate to avoid esophageal ulceration. Estrogen hormone replacement therapy ± vitamin D also has proven valuable for slowing bone resorption in menopause, and increases in bone mass have been reported for combinations of estrogens with alendronate.

9. **Answer: A.** Octreotide is a somatostatin analog that is effective for carcinoid and other secretory GI tumors. It has been used to varying degrees in other forms of secretory diarrhea such as chemotherapy-induced and the diarrhea of HIV and diabetes. Octreotide has proven to be of major importance in the management of acromegaly and can significantly decrease the levels of growth hormone.

PART IX

Anticancer Drugs

Anticancer Drugs 1

Learning Objectives

- ❏ Define the mechanisms of anti-cancer drugs
- ❏ Demonstrate an understanding of the toxicity of anticancer drugs

PRINCIPLES

Log-Kill Hypothesis

Cytotoxic actions of anticancer drugs follow first-order kinetics: They kill a fixed percentage of tumor cells, not a fixed number → one rationale for drug combinations.

Growth Fraction

Cytotoxic drugs are more effective against tumors that have a high growth fraction (large percentage actively dividing). Normal cells with high growth fraction (e.g., bone marrow) are also more sensitive to anticancer drugs.

DRUGS AND DRUG PROPERTIES

Cell-Cycle Specificity

- Drugs that act specifically on phases of the cell cycle are called cell-cycle specific (CCS) and are more effective in tumors with high-growth fraction (leukemias, lymphomas).
- Drugs that are cell-cycle nonspecific (many bind to and damage DNA) can be used in tumors with low-growth fraction, as well as tumors with high-growth fraction.

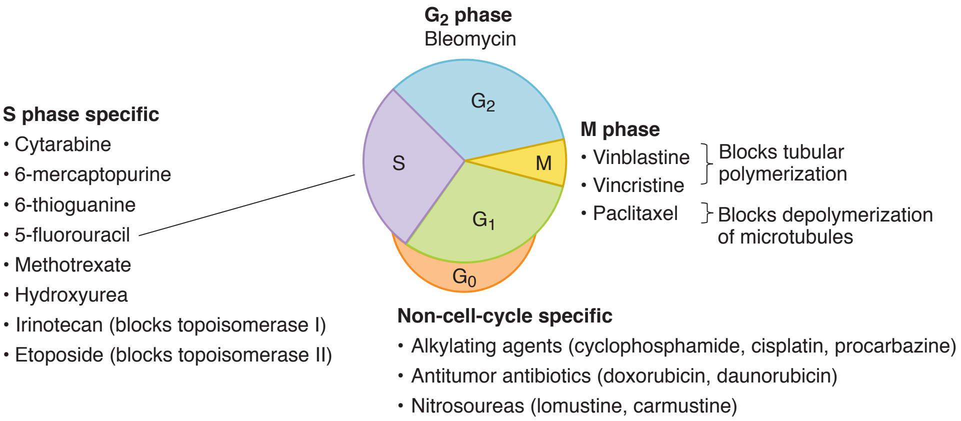


Figure IX-1-1. Cell-Cycle Specificity of Anticancer Drugs

Cancer Drug Characteristics

High-Yield

Table IX-1-1. Characteristics of Important Anticancer Drugs

Drug	Mechanism	Uses	Side Effects
Cyclophosphamide	Alkylating agent—attacks guanine N7—dysfunctional DNA	Non-Hodgkin, ovarian, breast cancer, neuroblastoma	BMS, hemorrhagic cystitis (**mesna,** traps acrolein and is protective)
Cisplatin	Alkylating agent—cross-links DNA strands	Testicular, ovarian, bladder, lung cancer	Nephrotoxicity (use **amifostine**); neurotoxicity (deafness)
Procarbazine	Alkylating agent	Hodgkin	BMS, leukemogenic
Doxorubicin	Intercalator, forms free radicals, inhibits topoisomerase	Hodgkin, breast, endometrial, lung, and ovarian cancers	BMS—delayed CHF (**dexrazoxane** is an iron-chelating agent preventing the formation of free radicals; it is not a free radical "trapper")
Methotrexate (CCS)	Antimetabolite—inhibits DHF reductase (S phase)	Leukemias, lymphomas, breast cancer; rheumatoid arthritis, psoriasis	BMS, **leucovorin** (folinic acid) rescue
5-Fluorouracil (CCS) Capecitabine (oral)	Pyrimidine antimetabolite (S phase) bioactivated to inhibit thymidylate synthetase	Breast, ovarian, head, and neck cancer—topical for basal cell cancer and keratoses; colorectal cancer	BMS
6-Mercaptopurine (CCS)	Purine antimetabolite (S phase) bioactivated by HGPR transferase	Acute lymphocytic leukemia; immunosuppression	BMS
Bleomycin (CCS)	Complexes with Fe and O_2 → DNA strand scission (G_2 phase)	Hodgkin, testicular, head, neck, skin cancer	Pneumonitis, pulmonary fibrosis
Vinblastine (CCS) Vincristine	↓ Microtubular polymerization—spindle poisons (M phase)	Vinblastine—Hodgkin, testicular cancer, Kaposi Vincristine—Hodgkin, leukemias, Wilms	BMS Neurotoxicity
All-trans retinoic acid (ATRA)	Differentiating agent, promotes differentiation of promyelocytes	Acute myelogenous leukemia (AML), M3	"Differentiation syndrome" with respiratory distress, pleural and pericardial effusions, CNS symptoms

Definition of abbreviations: BMS, bone marrow suppression; CCS, cell-cycle specific

Targeted Cancer Therapy

High-Yield

Table IX-1-2. Targeted Anticancer Drugs

Drug	Target
Imatinib	BCR-ABL
Cetuximab	ErbB1
Trastuzumab	ErbB2 (HER2/neu)
Bevacizumab	VEGF-A
Sorafenib	RAF kinase

Toxicity of Anticancer Drugs

High-Yield

Table IX-1-3. Other Dose-Limiting or Distinctive Toxicities

Toxicity	Drug(s)
Renal	Cisplatin,* methotrexate
Pulmonary	Bleomycin,* busulfan, procarbazine
Cardiac	Doxorubicin, daunorubicin
Neurologic	Vincristine,* cisplatin
Immunosuppressive	Cyclophosphamide, methotrexate
Other	Cyclophosphamide (hemorrhagic cystitis); procarbazine (leukemia); asparaginase* (pancreatitis)

*Less BMS: "marrow sparing"

Rapidly proliferating cells such as the bone marrow, gastrointestinal tract mucosa, hair follicles, and gonads are the most sensitive to cytotoxic drugs. Most often **bone marrow suppression** (BMS) is dose-limiting.

Cytokines

Anticancer drug dosage is usually carefully titrated to avoid excessive neutropenia (granulocytes <500/mm^3) and thrombocytopenia (platelets <20,000/mm^3). Colony-stimulating factors, erythropoietin, and thrombopoietin can be supportive →↓ infections and need for antibiotics.

Clinical Correlate

Thymineless Death of Cells

Flucytosine (FC) and 5-fluorouracil (5-FU) are bioactivated to 5-fluorodeoxyuridine (5-FdUMP), which inhibits thymidylate synthetase → "thymineless death" of fungal cells (FC) or neoplastic cells (5-FU).

Table IX-1-4. Clinical Uses of Cytokines

Cytokine	Clinical Uses
Aldesleukin (IL-2)	↑ lymphocyte differentiation and ↑ NKs: used in renal cell cancer and metastatic melanoma
Interleukin-11	↑ platelet formation: used in thrombocytopenia
Filgrastim (G-CSF)	↑ granulocytes: used for marrow recovery
Sargramostim (GM-CSF)	↑ Granulocytes and macrophages—used for marrow recovery
Erythropoietin	Anemias, especially associated with renal failure
Thrombopoietin	Thrombocytopenia

Recall Question

Which of the following medications has the major side effect of nephrotoxicity rather than bone marrow suppression?

A. Doxorubicin

B. Cisplatin

C. Methotrexate

D. Vinblastine

Answer: B

Anticancer Drug Practice Questions

2

PRACTICE QUESTIONS

1. Which of the following chemotherapeutic drugs inhibits the polymerization of microtubules but is not associated with causing bone marrow suppression?

    A. Cyclophosphamide
    B. Cisplatin
    C. 5-Fluorouracil
    D. Vinblastine
    E. Vincristine

2. A patient with non-Hodgkin lymphoma is to be started on the CHOP regimen, which consists of cyclophosphamide, doxorubicin, vincristine, and prednisone. Which one of the following agents is most likely to be protective against the toxicity of doxorubicin?

    A. Amifostine
    B. Dexrazoxane
    C. Leucovorin
    D. Mesna
    E. Vitamin C

3. A drug used in a chemotherapy regimen works by complexing with iron and oxygen to promote DNA strand breaks. While on this drug the patient must be monitored closely due to pulmonary side effects. In what phase of the cell cycle does this drug work?

    A. G_1
    B. S
    C. G_2
    D. M
    E. This drug is not cell-cycle dependent.

4. Resistance to which anticancer drug, used in leukemias, lymphomas, and breast cancer, is associated with increased production of dihydrofolate reductase?

   A. Doxorubicin
   B. Vinblastine
   C. 6-MP
   D. Cytarabine
   E. Methotrexate

5. A patient undergoing cancer chemotherapy has an increase in urinary frequency with much discomfort. No specific findings are apparent on physical examination. Laboratory results include hematuria and mild leukopenia, but no bacteria or crystalluria. If the symptoms experienced by the patient are drug related, what is the most likely cause?

   A. Cyclophosphamide
   B. 5-FU
   C. Methotrexate
   D. Prednisone
   E. Tamoxifen

ANSWERS AND EXPLANATIONS

1. **Answer: E.** Only two of the drugs listed do not cause bone marrow suppression: cisplatin and vincristine. Only two of the drugs listed inhibit microtubule polymerization: vinblastine and vincristine. The drug that fits both categories is vincristine. Patients on vincristine should be monitored for neurotoxicity, especially peripheral neuropathies.

2. **Answer: B.** Dexrazoxane is an iron-chelating agent that prevents the formation of free radicals and reduces the cardiotoxicity of anthracyclines such as doxorubicin. Amifostine is protective of nephrotoxicity caused by cisplatin. Folinic acid (leucovorin) reduces the toxicity of methotrexate because it provides an active form of folate to normal (nonneoplastic) cells, resulting in "leucovorin rescue." Mesna, which inactivates acrolein, is available for protection against hemorrhagic cystitis in patients treated with cyclophosphamide.

3. **Answer: C.** It helps to know which anticancer drugs are cell-cycle specific and which have characteristic toxicities. Bleomycin forms a complex with iron and oxygen and promotes DNA strand breaks. Its major side effects are pulmonary toxicities including pneumonitis and fibrosis. It acts acting mainly in the G_2 phase of the cell-cycle.

4. **Answer: E.** Methotrexate is a widely-used chemotherapy drug that is also commonly used in moderate to severe rheumatoid arthritis. It inhibits the enzyme dihydrofolate reductase (DHFR) thereby reducing the synthesis of tetrahydrofolate and thus inhibiting DNA synthesis. Resistance occurs when cancer cells upregulate DHFR or alter the binding of methotrexate to DHFR.

5. **Answer: A.** These symptoms are those of a mild case of hemorrhagic cystitis. Bladder irritation with hematuria is a fairly common complaint of patients treated with cyclophosphamide. It appears to be due to acrolein, a product formed when cyclophosphamide is bioactivated by liver P450 to form cytotoxic metabolites. Mesna is the antidote used to detoxify acrolein and protect against hemorrhagic cystitis.

PART X

Immunopharmacology

Immunopharmacology 1

Learning Objectives

- ❑ Answer mechanism and side effect questions about cyclosporine, tacrolimus, mycophenolate, azathioprine, and anti-D immunoglobulin
- ❑ List the most commonly used monoclonal antibodies
- ❑ Explain information related to cytokines (recombinant forms)

IMMUNOSUPPRESSANTS

Cyclosporine and Tacrolimus

High-Yield

- Mechanism of action:
  - Bind to cyclophilin (cyclosporine) or FK-binding protein (tacrolimus) →↓ calcineurin (cytoplasmic phosphatase) →↓ activation of T-cell transcription factors →↓ IL-2, IL-3, and interferon-γ
- Uses:
  - Cyclosporine is DOC for organ or tissue transplantation (+/– mycophenolate, +/– steroids, +/– cytotoxic drugs)
  - Tacrolimus used alternatively to cyclosporine in renal and liver transplants
- Side effects: nephrotoxicity (both), gingival overgrowth (cyclosporine)

Mycophenolate

An inhibitor of de novo synthesis of purines, has adjunctive immunosuppressant actions, permitting dose reductions of cyclosporine to limit toxicity.

Azathioprine

Immunosuppressant converted to 6-mercaptopurine (same properties as 6-MP)

Anti-D Immunoglobulin

- Human IgG antibodies to red cell D antigen (rhesus antigen)
- Uses: administer to Rh-negative mother within 72 hours of Rh-positive delivery to prevent hemolytic disease of newborn in subsequent pregnancy

Monoclonal Antibodies

Table X-1-1. Clinical Uses of Monoclonal Antibodies

Mab	Clinical Uses
Abciximab	Antiplatelet: antagonist of IIb/IIIa receptors
Infliximab	Rheumatoid arthritis and Crohn disease: binds TNF
Adalimumab	Rheumatoid arthritis and Crohn disease: binds TNF
Trastuzumab	Breast cancer: antagonist to ERB-B2 (Her 2/neu)
Idarucizumab	Rapid reversal of dabigatran
Muromonab	Kidney transplant: blocks allograft rejection
Palivizumab	Respiratory syncytial virus: blocks RSV protein
Rituximab	Non-Hodgkin lymphoma: binds to surface protein

Cytokines (Recombinant Forms)

Table X-1-2. Clinical Uses of Interferons

Interferon-α	Hepatitis B and C, leukemias, melanoma
Interferon-β	Multiple sclerosis
Interferon-γ	Chronic granulomatous disease →↑ TNF

Immunopharmacology Practice Questions

2

PRACTICE QUESTIONS

1. A patient is treated with an immunosuppressant drug following a liver transplant. The drug is known to bind to cyclophilin and inhibit the actions of calcineurin. For what drug toxicity should this patient be monitored?

   A. Pulmonary fibrosis
   B. Hypotension
   C. Hypoglycemia
   D. Nephrotoxicity
   E. CHF

2. Which one of the following agents has utility in the management of acute coronary syndromes such as unstable angina?

   A. Abciximab
   B. Interferon-α
   C. Aldesleukin
   D. Filgrastim
   E. Trastuzumab

ANSWERS AND EXPLANATIONS

1. **Answer: D.** This patient is being treated with cyclosporin, a drug that binds to cyclophilin and inhibits calcineurin. As a result, the transcription of various T-cells factors such as IL-2, IL-3, and Interferon-γ are inhibited. Cyclosporin is associated with nephrotoxicity, gingival hyperplasia, hyperglycemia, hypertension, and hirsutism.

2. **Answer: A.** Abciximab is an antibody-based drug that targets glycoprotein IIb/IIIa receptors. Binding of the drug to these receptors results in decreased platelet aggregation by preventing the cross-linking reaction. It is useful in acute coronary syndromes such as unstable angina and post-angioplasty.

PART XI

Toxicology

Toxicology 1

Learning Objectives

- ❏ Describe common toxic syndromes
- ❏ Explain information related to heavy metal poisoning and chelation therapy
- ❏ List commonly used antidotes
- ❏ Demonstrate understanding of natural medicinals

TOXICOLOGY

Common Toxic Syndromes

High-Yield

Table XI-1-1. Signs, Symptoms, and Interventions or Antidotes for Common Toxic Syndromes

Compound	Signs and Symptoms	Interventions and Antidotes
AChE inhibitors	Miosis, salivation, sweats, GI cramps, diarrhea, muscle twitches → seizures, coma, respiration failure	Respiratory support; atropine + pralidoxime (for irreversible AChE inhibitors)
Atropine and muscarinic blockers	↑ HR, ↑ BP, hyperthermia (hot, dry skin), delirium, hallucinations, mydriasis	Control cardiovascular symptoms and hyperthermia + physostigmine (crosses blood–brain barrier)
Carbon monoxide (>10% carboxyHb)	Nausea and vomiting, dyspnea with hyperventilation, mydriasis, vertigo; cardiovascular signs prominent, ↓ BP, syncope, ↑ HR, arrhythmias	Hyperbaric O_2 and decontamination (humidified 100% O_2 okay in mild overdose)
CNS stimulants	Anxiety/agitation, hyperthermia (warm, sweaty skin), mydriasis, ↑ HR, ↑ BP, psychosis, seizures	Control cardiovascular symptoms, hyperthermia, and seizures— +/− BZs or antipsychotics
Opioid analgesics	Lethargy, sedation, ↓ HR, ↓ BP, hypoventilation, miosis, coma, respiration failure	Ventilatory support; naloxone at frequent intervals
Salicylates (ASA)*	Confusion, lethargy, hyperventilation, hyperthermia, dehydration, hypokalemia, acidosis, seizures, coma	Correct acidosis and electrolytes: urinary alkalinization, possible hemodialysis
Sedative-hypnotics and ethanol	Disinhibition (initial), lethargy, ataxia, nystagmus, stupor, coma, hypothermia, respiratory failure	Ventilatory support: flumazenil if BZs implicated
SSRIs	Agitation, confusion, hallucination, muscle rigidity, hyperthermia, ↑ HR, ↑ BP, seizures	Control hyperthermia and seizures: possible use of cyproheptadine, antipsychotics, and BZs
Tricyclic antidepressants	Mydriasis, hyperthermia (hot, dry skin), 3 Cs (convulsions, coma, and cardiotoxicity) → arrhythmias	Control seizures and hyperthermia, correct acidosis and possible arrhythmias

*More details in antiinflammatory section

Heavy Metal Poisoning

High-Yield

Signs and symptoms are distinctive but usually result from inhibition of –SH groups on enzymes and regulatory proteins.

Table XI-1-2. Signs, Symptoms, and Interventions or Antidotes for Heavy Metal Poisoning

Metals and Source	Signs and Symptoms	Interventions and Antidotes
Arsenic (wood preservatives, pesticides, ant poisons)	**Acute:** gastroenteritis, hypotension, metabolic acidosis, garlic breath, "rice water" stools, torsades, seizures **Chronic:** pallor, skin pigmentation (raindrop pattern), alopecia, stocking glove neuropathy, myelosuppression	Activated charcoal, **dimercaprol** **Penicillamine** or succimer
Iron (medicinal for anemias and prenatal supplements)	**Acute** (mainly children): severe GI distress → necrotizing gastroenteritis with hematemesis and bloody diarrhea, dyspnea, shock, coma	Gastric aspiration + carbonate lavage, **deferoxamine** IV
Lead (tap water, leaded paint chips, herbal remedies, gas sniffing, glazed kitchenware, etc.)	**Acute:** nausea and vomiting, GI distress and pain, malaise, tremor, tinnitus, paresthesias, encephalopathy (red or black feces) **Chronic:** multisystem effects: anemia (↓ heme synthesis), neuropathy (wrist drop), nephropathy (proteinuria, failure), hepatitis, mental retardation (from pica), ↓ fertility and ↑ stillbirths	Decontamination—gastric lavage + **dimercaprol** (severe) or EDTA or succimer (penicillamine if unable to use dimercaprol or succimer) Children: **succimer** PO
Mercury (elemental in instruments); salts used in amalgams, batteries, dyes, electroplating, fireworks, photography	**Acute:** vapor inhalation: chest pain, dyspnea, pneumonitis **Acute:** inorganic salt ingestion: hemorrhagic gastroenteritis, acute tubular necrosis, shock **Chronic:** organic Hg—CNS effects, ataxia, paresthesias, auditory and visual loss, loosening of teeth	**Succimer** PO or **dimercaprol (IM)** Activated charcoal for oral ingestion, then support with succimer PO or dimercaprol (***not*** IV) → causes redistribution of Hg to the CNS →↑ neurotoxicity

Antidotes

High-Yield

Table XI-1-3. Antidotes

Antidote	Type of Poisoning
Acetylcysteine	Acetaminophen
Atropine + pralidoxime (for irreversible AChE inhibitors)	AChE inhibitors—physostigmine, neostigmine, and pyridostigmine; organophosphates, including insecticides, such as malathion and parathion
Deferoxamine	Iron and iron salts
Digoxin immune F(ab)	Digoxin
Dimercaprol (BAL)	Arsenic, gold, mercury, lead; oral succimer for milder lead and mercury toxicity
EDTA	Backup in lead poisoning, then for rarer toxicities (Cd, Cr, Co, Mn, Zn)
Esmolol	Theophylline, beta agonists
Ethanol, fomepizole	Methanol or ethylene glycol
Flumazenil	Benzodiazepines, zolpidem, zaleplon
Naloxone	Opioid analgesics
Oxygen	Carbon monoxide
Penicillamine	Copper (e.g., Wilson's disease), iron, lead, mercury
Physostigmine	Anticholinergics: atropine, antihistamine, antiparkinsonian—*not* tricyclics
Protamine	Heparins
Vitamin K	Warfarin and coumarin anticoagulants
Activated charcoal	Nonspecific: all oral poisonings except Fe, CN, Li, solvents, mineral acids, or corrosives

Recall Question

Which of the following is the drug of choice for iron poisoning?

A. Deferoxamine

B. Dimercaprol (BAL)

C. EDTA

D. Penicillamine

Answer: A

Natural Medicinals

"Natural" medicinals are available without prescription and are considered to be nutritional supplements rather than drugs. Herbal (botanic) products are marketed without FDA review of safety and efficacy, and there are no requirements governing the purity or the chemical identities of constituents.

Evidence supporting the clinical effectiveness of herbal products is commonly incomplete.

Table XI-1-4. Characteristics of Selected Herbals

Name	Medicinal Use(s)	Possible Mechanism(s)	Side Effects
Echinacea	↓ Cold symptoms	↑ ILs and TNF	GI distress, dizziness, headache
Garlic	Hyperlipidemias, cancer (evidence is weak)	Inhibits HMG-CoA reductase and ACE	Allergies, hypotension, antiplatelet actions; use caution when used with anticoagulants
Gingko	Intermittent claudication; Alzheimer disease (evidence is weak)	Antioxidant, free radical scavenger, ↑ NO	Anxiety, GI distress, insomnia, antiplatelet actions; **use caution when used with anticoagulants**
Ginseng	Possible ↑ in mental and physical performance (evidence is weak)	Unknown	Insomnia, nervousness, hypertension, mastalgia, vaginal bleeding
Saw palmetto	Symptomatic treatment of BPH	5α-reductase inhibitor and androgen receptor antagonist	GI pain, decreased libido, headache, hypertension
St. John's wort	Depressive disorder (variable evidence for clinical efficacy)	May enhance brain 5HT functions	Major drug interactions: **serotonin syndrome** with SSRIs; induces P450, leading to ↓ effects of multiple drugs

Table XI-1-5. Purified Nutritional Supplements

Name	Pharmacology	Side Effects
Dehydroepiandrosterone (DHEA)	Androgen precursor advocated for treatment of AIDS (↑CD4 in women), Alzheimer disease and “aging,” diabetes, hypercholesterolemia, and SLE (↓in symptoms and “flare-ups” in women)	• **Women:** androgen-ization and concern regarding CV disease and breast cancer • **Men:** feminization in young and concern in elderly regarding BPH and cancer
Melatonin	Serotonin metabolite used for “jet-lag” and sleep disorders	• Drowsiness, sedation, headache • Contraindicated in pregnancy, in women trying to conceive (↓LH), and in nursing mothers (↓prolactin)

Toxicology Practice Questions

2

PRACTICE QUESTIONS

1. Chronic ingestion of lead-based paint chips will result in which of the following?

   A. Garlic breath
   B. Changes in skin pigmentation
   C. Accumulation of δ-aminolevulinate and inhibition of heme synthesis
   D. Auditory and visual loss
   E. Interstitial pneumonitis and neurological effects

2. A 3-year-old child was brought to the ER following the ingestion of several pills. The child is suffering from severe gastrointestinal discomfort and has thrown up twice, each time producing a bloody vomitus. Questioning of the mother reveals the child got into the mother's old prenatal vitamins. What antidote should be given?

   A. Dimercaprol
   B. Deferoxamine
   C. EDTA
   D. Penicillamine
   E. Succimer

ANSWERS AND EXPLANATIONS

1. **Answer: C.** Chronic poisoning with lead will result in a multitude of effects including inhibition of heme synthesis and accumulation of δ-aminolevulinate in the plasma. Arsenic poisoning is associated with garlic breath and changes in skin pigmentation. Organic mercury causes auditory and visual loss and loosening of the teeth, while inhaled mercury vapor can cause interstitial pneumonitis and neurological effects.

2. **Answer: B.** The child is suffering from iron poisoning. Deferoxamine chelates iron and is the antidote in iron poisoning. The other choices are all metal chelators with utility in other types of heavy metal poisoning. Dimercaprol is useful for a variety of metals including lead, arsenic, and mercury. EDTA is a back-up in lead poisoning. Penicillamine is useful in copper poisoning, and succimer is preferred for lead poisoning in kids.

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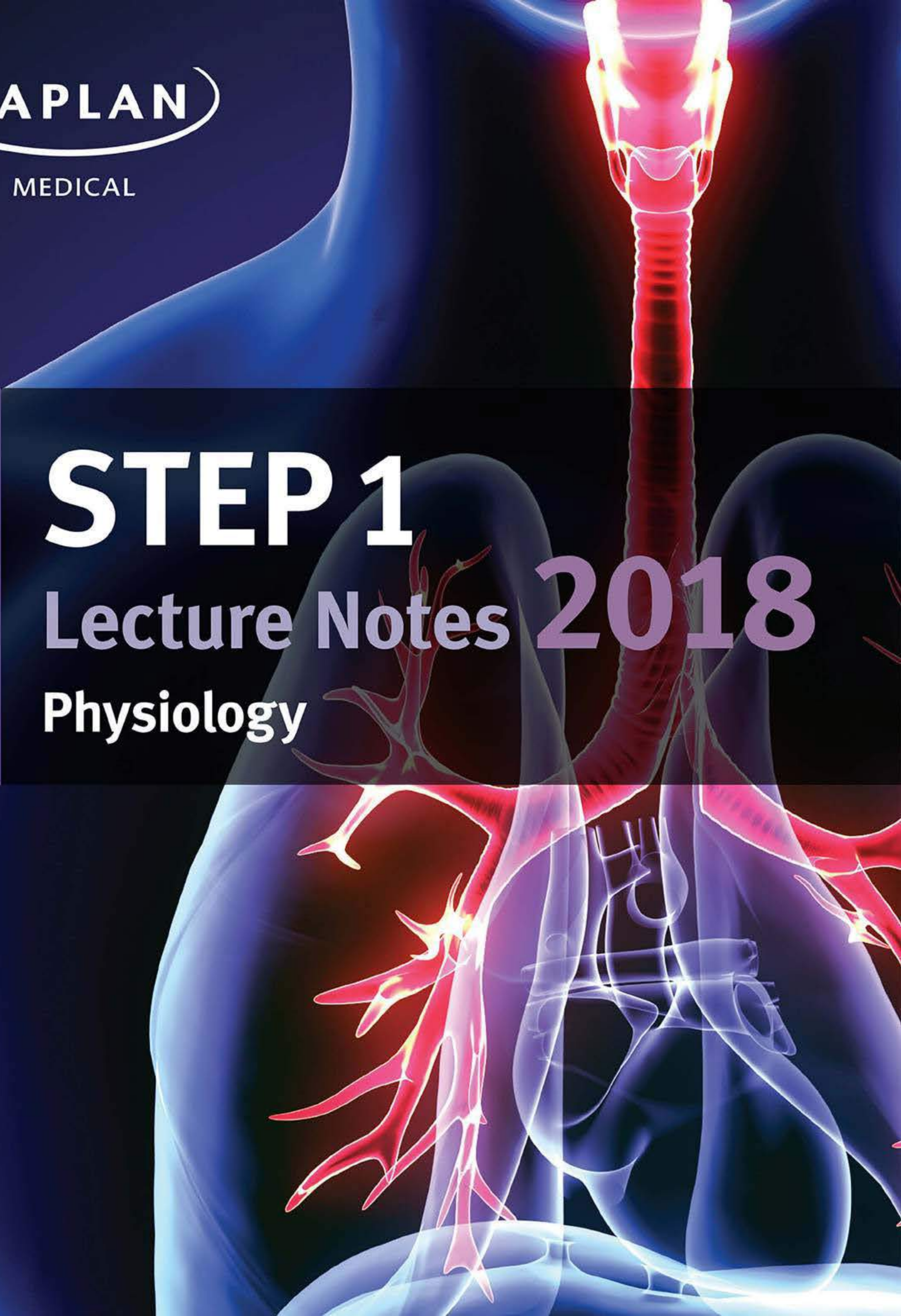

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PART I

Fluid Distribution and Edema

Fluid Distribution and Edema 1

Learning Objectives

- ❏ Interpret scenarios on distribution of fluids within the body
- ❏ Answer questions about review and integration
- ❏ Use knowledge of microcirculation
- ❏ Interpret scenarios on edema (pathology integration)
- ❏ Interpret scenarios on volume measurement of compartments

DISTRIBUTION OF FLUIDS WITHIN THE BODY

Total Body Water

- Intracellular fluid (ICF): approximately 2/3 of total body water
- Extracellular fluid (ECF): approximately 1/3 of total body water
- Interstitial fluid (ISF): approximately 3/4 of the extracellular fluid
- Plasma volume (PV): approximately 1/4 of the extracellular fluid
- Vascular compartment: contains the blood volume which is plasma and the cellular elements of blood, primarily red blood cells

It is important to remember that membranes can serve as barriers. The 2 important membranes are shown below. The **cell membrane** is a relative barrier for Na^+, while the **capillary membrane** is a barrier for plasma proteins.

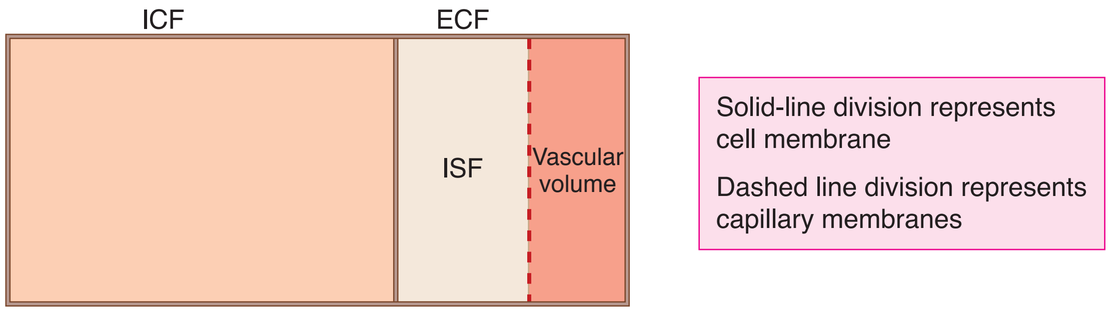


Figure I-1-1. Body Compartments

Osmosis

The distribution of fluid is determined by the osmotic movement of water. Osmosis is the diffusion of water across a semipermeable or selectively permeable membrane. Water diffuses from a region of higher water concentration to a region of lower water concentration. The concentration of water in a solution is determined by the concentration of solute; the greater the solute concentration, the lower the water concentration.

The osmotic properties are defined by:

- **Osmolarity:**

  mOsm (milliosmoles)/L = concentration of particles per liter of solution

- **Osmolality:**

  mOsm/kg = concentration of particles per kg of solvent (water being the germane one for physiology/medicine)

It is the **number of particles** that is crucial, as shown below. There are 2 compartments separated by a membrane that is permeable to water but not to solute.

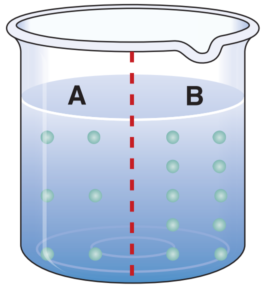


Figure I-1-2. Osmosis

Side B has the greater concentration of solute (circles) and thus a lower water concentration than side A. As a result, water diffuses from A to B, and the height of column B rises, and that of A falls.

If a solute does not easily cross a membrane, then it is an **"effective" osmole** for that compartment, i.e., it creates an osmotic force for water. For example, plasma proteins do not easily cross the capillary membrane, so they serve as effective osmoles for the vascular compartment.

Sodium does not easily penetrate the cell membrane, but it does cross the capillary membrane, thus it is an effective osmole for the extracellular compartment.

Extracellular Solutes

A basic metabolic profile/panel (BMP) includes the common labs provided from a basic blood draw, often with normal values for the solutes.

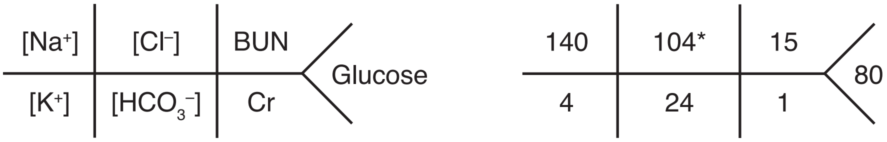


Figure I-1-3. Basic Metabolic Profile/Panel

*Value provided for chloride is the one most commonly used, but it can vary depending upon the lab

Osmolar Gap

The osmolar gap is the difference between the **measured** osmolality and the **estimated** osmolality using the equation below. Using the data from the BMP, we can estimate the extracellular osmolality using the following formula:

$$\textbf{ECF estimated osmolality} = 2\left(\mathbf{Na^+}\right)\mathbf{mEq/L} + \frac{\textbf{glucose mg \%}}{\mathbf{18}} + \frac{\textbf{urea mg \%}}{\mathbf{2.8}}$$

The basis of this calculation is:

- Na^+ is the most abundant osmole of the extracellular space.
- Na^+ is doubled because it is a positive charge and thus for every positive charge there is a negative charge (chloride being the most abundant, but not the only one).
- The 18 and 2.8 are converting glucose and BUN into their respective osmolarities (their units of measurement are mg/dL).

Determining the osmolar gap (normal ≤ 15) is helpful for narrowing the differential diagnosis. While many things can elevate the osmolar gap, some of the more common are ethanol, methanol, ethylene glycol, acetone, and mannitol. Thus, an inebriated patient has an elevated osmolar gap.

Graphical Representation of Body Compartments

It is important to understand how body osmolality and the intracellular and extracellular volumes change in clinically relevant situations. One way to present this information is shown below. The y axis is solute concentration or osmolality. The x axis is the volume of intracellular (2/3) and extracellular (1/3) fluid.

If the solid line represents the control state, the dashed lines show a decrease in osmolality and extracellular volume but an increase in intracellular volume.

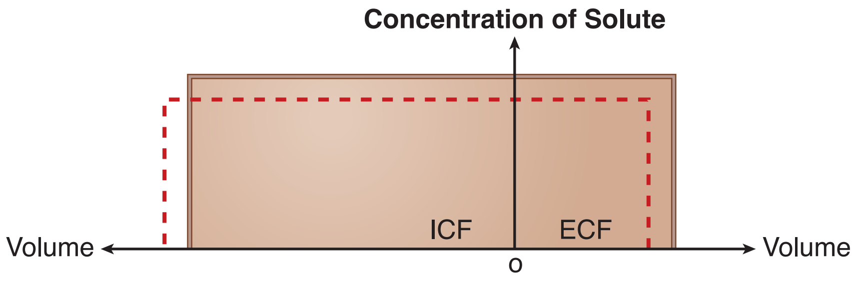


Figure I-1-4. Darrow-Yannet Diagram

Note

Normal values will be provided on the exam so there is no need to memorize these numbers. However, knowing them can be useful for time management.

Ranges

Na^+: 136–145 mEq/L

K^+: 3.5–5.0 mEq/L

Cl^-: 100–106 mEq/L

HCO_3^-: 22–26 mEq/L

BUN: 8–25 mg/dl

Cr (creatinine): 0.8–1.2 mg/dl

Glucose: 70–100 mg/dl

- **Extracellular volume** always enlarges when there is a net gain of fluid by the body. Extracellular volume always decreases when there is a net loss of body fluid.
- **Concentration of solutes** is equivalent to body osmolality. At steady-state, the intracellular concentration of water equals the extracellular concentration of water (cell membrane is not a barrier for water). Thus, the intracellular and extracellular osmolalities are the same.
- **Intracellular volume** varies with the effective osmolality of the extracellular compartment. Solutes and fluids enter and leave the extracellular compartment first (sweating, diarrhea, fluid resuscitation, etc.). Intracellular volume is only altered if extracellular osmolality changes.
- If ECF osmolality increases, cells lose water and shrink. If ECF osmolality decreases, cells gain water and swell.

Below are 6 Darrow-Yannet diagrams illustrating changes in volume and/or osmolality. Examine the alterations, trying to determine what occurred and how. Consider whether the change represents net water and/or solute gain or loss.

Indicate, too, how the situation would likely occur from a clinical perspective, i.e., the patient is hemorrhaging, drinking water, consuming excess salt, etc.

Changes in volume and concentration (dashed lines)

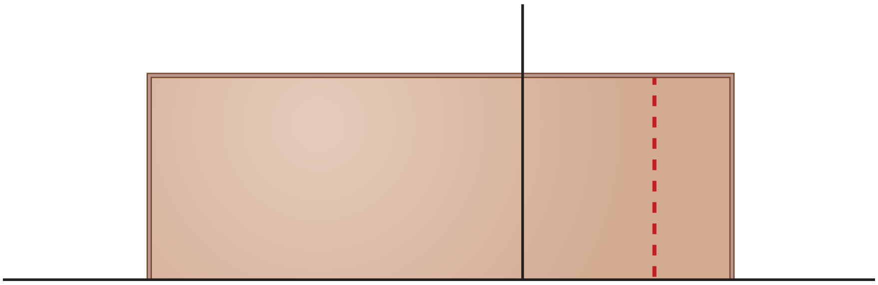
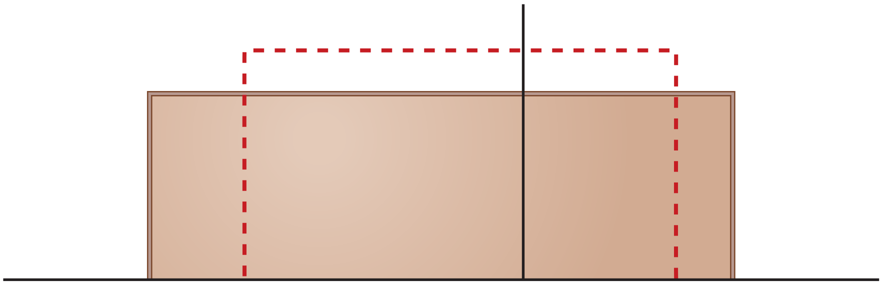


Figure I-1-5.



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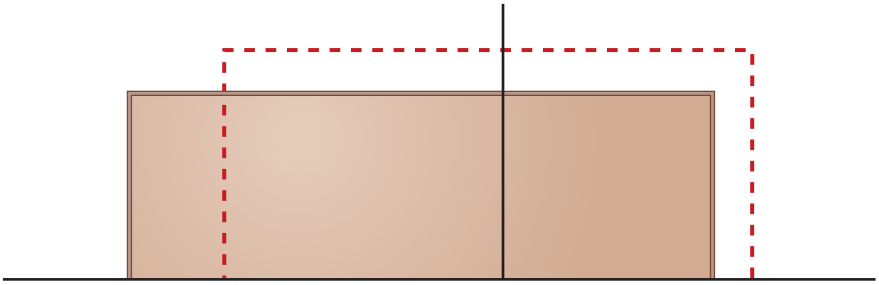

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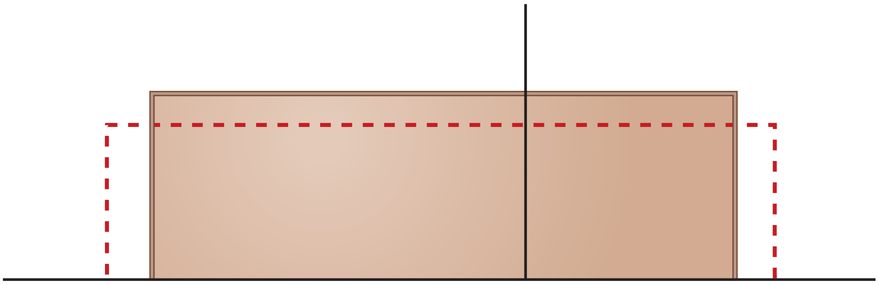


Figure I-1-8.

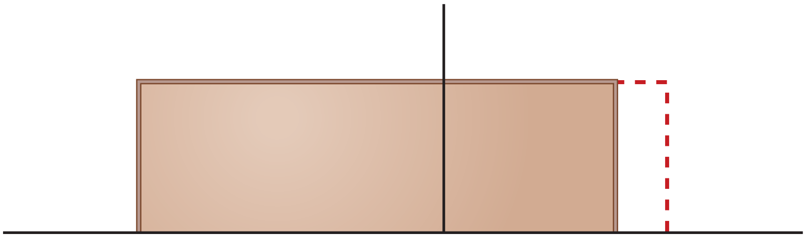


Figure I-1-9.

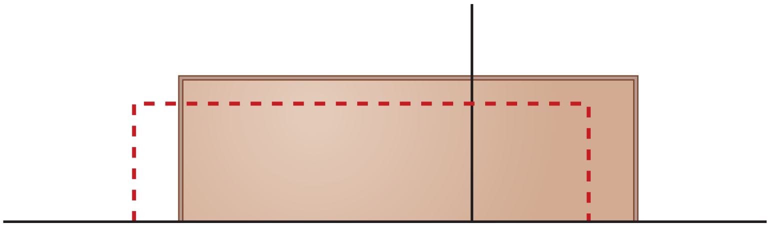


Figure I-1-10.

Explanations

Figure I-1-5: Patient shows loss of extracellular volume with no change in osmolality. Since extracellular osmolality is the same, then intracellular volume is unchanged. This represents an **isotonic fluid loss (equal loss of fluid and osmoles)**. Possible causes are hemorrhage, isotonic urine, or the immediate consequences of diarrhea or vomiting.

Figure I-1-6: Patient shows loss of extracellular and intracellular volume with rise in osmolality. This represents a **net loss of water (greater loss of water than osmoles)**. Possible causes are inadequate water intake or sweating. Pathologically, this could be hypotonic water loss from the urine resulting from diabetes insipidus.

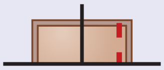
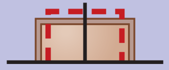
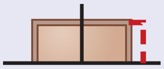
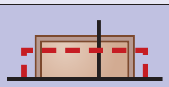
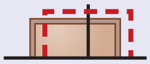
Figure I-1-7: Patient shows gain of extracellular volume, increase in osmolality, and a decrease in intracellular volume. The rise in osmolality shifted water out of the cell. This represents a **net gain of solute (increase in osmoles greater than increase in water)**. Possible causes are ingestion of salt, hypertonic infusion of solutes that distribute extracellularly (saline, mannitol), or hypertonic infusion of colloids. Colloids, e.g. dextran, don't readily cross the capillary membrane and thus expand the vascular compartment only (vascular is part of extracellular compartment).

Figure I-1-8: Patient shows increase in extracellular and intracellular volumes with a decrease in osmolality. The fall in osmolality shifted water into the cell. Thus, this represents **net gain of water (more water than osmoles)**. Possible causes are drinking significant quantities of water (could be pathologic primary polydipsia), drinking significant quantities of a hypotonic fluid, or a hypotonic fluid infusion (saline, dextrose in water). Pathologically this could be abnormal water retention such as that which occurs with syndrome of inappropriate ADH.

Figure I-1-9: Patient shows increase in extracellular volume with no change in osmolality or intracellular volume. Since extracellular osmolality didn't change, then intracellular volume is unaffected. This represents a **net gain of isotonic fluid (equal increase fluid and osmoles)**. Possible causes are isotonic fluid infusion (saline), drinking significant quantities of an isotonic fluid, or infusion of an isotonic colloid. Pathologically this could be the result of excess aldosterone. Aldosterone is a steroid hormone that causes Na^+ retention by the kidney. At first glance one would predict excess Na^+ retention by aldosterone would increase the concentration of Na^+ in the extracellular compartment. However, this is rarely the case because water follows Na^+, and even though the total body mass of Na^+ increases, its concentration doesn't.

Figure I-1-10: Patient shows decrease in extracellular volume and osmolality with an increase in intracellular volume. The rise in intracellular volume is the result of the decreased osmolality. This represents a **net loss of hypertonic fluid (more osmoles lost than fluid)**. The only cause to consider is the pathologic state of adrenal insufficiency. Lack of mineralocorticoids, e.g., aldosterone causes excess Na^+ loss.

Table I-1-1. Volume Changes and Body Osmolarity Following Changes in Body Hydration

	ECF Volume	Body Osmolarity	ICF Volume	D-Y Diagram
Loss of isotonic fluid Hemorrhage Diarrhea Vomiting	↓	no change	no change	
Loss of hypotonic fluid Dehydration Diabetes insipidus Alcoholism	↓	↑	↓	
Gain of isotonic fluid Isotonic saline	↑	no change	no change	
Gain of hypotonic fluid Hypotonic saline Water intoxication	↑	↓	↑	
Gain of hypertonic fluid Hypertonic saline Hypertonic mannitol	↑	↑	↓	

ECF = extracellular fluid; ICF = intracellular fluid; D-Y = Darrow-Yannet

Recall Question

Which of the following volume changes would most likely be seen in a 38-year-old man who is lost and dehydrated in a desert?

A. Loss of isotonic fluid with ECF volume contraction, no change in total body osmolarity, no change in ICF volume

B. Loss of hypotonic fluid with ECF volume contraction, increase in total body osmolarity, ICF volume contraction

C. Loss of hypotonic fluid with ECF volume contraction, no change in total body osmolarity, no change in ICF volume

D. Loss of hypertonic fluid with ECF volume contraction, decrease in total body osmolarity, increase in ICF volume

E. Loss of hypertonic fluid with ECF volume expansion, decrease in total body osmolarity, decrease in ICF volume

Answer: B

REVIEW AND INTEGRATION

Let’s review 2 important hormones involved in volume regulation: aldosterone and anti-diuretic hormone. These are also covered in greater detail in the Renal and Endocrine sections.

Aldosterone

One fundamental function of aldosterone is to increase sodium reabsorption in principal cells of the kidney. This reabsorption of sodium plays a key role in regulating extracellular volume.

Aldosterone also plays an important role in regulating plasma potassium and increases the secretion of this ion in principal cells.

The 2 primary factors stimulating aldosterone release are:

- **Plasma angiotensin II (Ang II)**
- **Plasma K^+**

Note

ADH secretion is primarily regulated by plasma osmolality and blood pressure/volume. However, it can also be stimulated by Ang II and corticotropin-releasing hormone (CRH).

This influence of **CRH** is particularly relevant to clinical medicine, because a variety of stresses (e.g., surgery) can increase ADH secretion.

Anti-Diuretic Hormone

Anti-diuretic hormone (ADH) (or arginine vasopressin [AVP]) stimulates water reabsorption in principal cells of the kidney via the V_2 receptor. By regulating water, ADH plays a pivotal role in regulating extracellular osmolality.

ADH also vasoconstricts arterioles (V_1 receptor) and thus can serve as a hormonal regulator of vascular tone.

The 2 primary regulators of ADH are:

- **Plasma osmolality** (**directly related**): an increase stimulates while a decrease inhibits
- **Blood pressure/volume** (**inversely related**): an increase inhibits while a decrease stimulates

Renin

Although renin is an enzyme, not a hormone, it is important in this discussion because it catalyzes the conversion of angiotensinogen to angiotensin I, which in turn is converted to Ang II by angiotensin converting enzyme (ACE). This is the renin-angiotensin-aldosterone system (RAAS).

The 3 primary regulators of renin are:

- **Perfusion pressure to the kidney (inversely related)**: an increase inhibits, while a decrease stimulates
- **Sympathetic stimulation to the kidney** (direct effect via β-1 receptors)
- **Na^+ delivery to the macula densa (inversely related)**: an increase inhibits, while a decrease stimulates

Negative Feedback Regulation

When examining the function and regulation of these hormones, one should see the feedback regulation. For example, aldosterone increases sodium reabsorption, which in turn increases extracellular volume. Renin is stimulated by reduced blood pressure (perfusion pressure to the kidney; reflex sympathetic stimulation). Thus, aldosterone is released as a means to compensate for the fall in arterial blood pressure.

Application

Given the above, review the previous Darrow-Yannet diagrams and predict what would happen to levels of each hormone in the various conditions.

Figure I-1-5: Loss of extracellular volume stimulates RAAS and ADH.

Figure I-1-6: Decreased extracellular volume stimulates RAAS. This drop in extracellular volume stimulates ADH, as does the rise in osmolarity. This setting would be a strong stimulus for ADH.

Figure I-1-7: The rise in extracellular volume inhibits RAAS. It is difficult to predict what will happen to ADH in this setting. The rise in extracellular volume inhibits, but the rise in osmolality stimulates, thus it will depend upon the magnitude of the changes. In general, osmolality is a more important factor, but significant changes in vascular volume/pressure can exert profound effects.

Figure I-1-8: The rise in extracellular volume inhibits RAAS and ADH. In addition, the fall in osmolality inhibits ADH.

Figure I-1-9: The rise in extracellular volume inhibits both.

Figure I-1-10: Although the only cause to consider is adrenal insufficiency, if this scenario were to occur, then the drop in extracellular volume stimulates RAAS. It is difficult to predict what happens to ADH in this setting. The drop in extracellular volume stimulates, but the fall in osmolality inhibits, thus it depends upon the magnitude of the changes.

MICROCIRCULATION

Filtration and Absorption

Fluid flux across the capillary is governed by the 2 fundamental forces that cause water flow:

- Hydrostatic force, which is simply the pressure of the fluid
- Osmotic (oncotic) force, which represents the osmotic force created by solutes that do not cross the membrane

Each force exists on both sides of the membrane. **Filtration** is the movement of fluid from the plasma into the interstitium, while **absorption** is movement of fluid from the interstitium into the plasma.

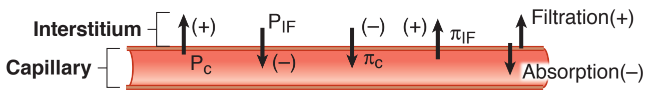


Figure I-1-11. Starling Forces

P = hydrostatic pressure

π = osmotic (oncotic) pressure (mainly proteins)

Forces for filtration

P_C = hydrostatic pressure (blood pressure) in the capillary

This is directly related to blood flow (regulated at the arteriole); venous pressure; and blood volume.

π_{IF} = oncotic (osmotic) force in the interstitium

This is determined by the concentration of protein in the interstitial fluid. Normally the small amount of protein that leaks to the interstitium is minor and is removed by the lymphatics. Under most conditions, this is not an important factor influencing the exchange of fluid.

Forces for absorption

π_C = oncotic (osmotic) pressure of plasma

This is the oncotic pressure of plasma solutes that cannot diffuse across the capillary membrane, i.e., the plasma proteins. Albumin, synthesized in the liver, is the most abundant plasma protein and thus the biggest contributor to this force.

P_{IF} = hydrostatic pressure in the interstitium

This pressure is difficult to determine. In most cases it is close to zero or negative (subatmospheric) and is not a significant factor affecting filtration versus reabsorption. It can become significant if edema is present or it can affect glomerular filtration in the kidney (pressure in Bowman's space is analogous to interstitial pressure).

Starling Equation

These 4 forces are often referred to as Starling forces. Grouping the forces into those that favor filtration and those that oppose it, and taking into account the properties of the barrier to filtration, the formula for fluid exchange is the following:

Qf: fluid movement

k: filtration coefficient

$$Qf = k\,[(P_c + \pi_{IF}) - (P_{IF} + \pi_C)]$$

The filtration coefficient depends upon a number of factors but for our purposes permeability is most important. As indicated below, a variety of factors can increase permeability of the capillary resulting in a large flux of fluid from the capillary into the interstitial space.

A positive value of Qf indicates net filtration; a negative value indicates net absorption. In some tissues (e.g., renal glomerulus), filtration occurs along the entire length of the capillary; in others (intestinal mucosa), absorption normally occurs along the whole length. In other tissues, filtration may occur at the proximal end until the forces equilibrate.

Lymphatics

The lymphatics play a pivotal role in maintaining a low interstitial fluid volume and protein content. Lymphatic flow is directly proportional to interstitial fluid pressure, thus a rise in this pressure promotes fluid movement out of the interstitium via the lymphatics.

The lymphatics also remove proteins from the interstitium. Recall that the lymphatics return their fluid and protein content to the general circulation by coalescing into the lymphatic ducts, which in turn empty into to the subclavian veins.

Review Questions

1. Given the following values, calculate a net pressure:
   P_C 25 mm Hg
   P_{IF} 2 mm Hg
   π_C 20 mm Hg
   π_{IF} 1 mm Hg
2. Calculate a net pressure if the interstitial hydrostatic pressure is –2 mm Hg.

Answers

1. +4 mm Hg
2. +8 mm Hg

EDEMA (PATHOLOGY INTEGRATION)

Edema is the accumulation of fluid in the interstitial space. It expresses itself in peripheral tissues in 2 forms:

- In **pitting edema** (classic, most common), pressing the affected area with a finger or thumb results in a visual indentation of the skin that persists for some time after the digit is removed. It generally responds well to diuretic therapy.
- In **non-pitting edema**, a persistent visual indentation is absent when pressing the affected area. This occurs when interstitial oncotic forces are elevated (proteins for example). It does not respond well to diuretic therapy.

Peripheral Edema

Significant alterations in the Starling forces, which then tip the balance toward filtration, increase capillary permeability (k), and/or interrupt lymphatic function, resulting in edema. Thus:

- **Increased capillary hydrostatic pressure (P_C)**: causes can include marked increase in blood flow (e.g., vasodilation in a given vascular bed); increasing venous pressure (e.g., venous obstruction or heart failure); and elevated blood volume, typically the result of Na^+ retention (e.g., heart failure).
- **Increased interstitial oncotic pressure (π_{IF}):** primarily caused by thyroid dysfunction (elevated mucopolysaccharides in the interstitium) but can be a result of lymphedema. Act as osmotic agents resulting in fluid accumulation and a non-pitting edema.

- **Decreased vascular oncotic pressure (π_C):** causes can include liver failure and nephrotic syndrome.
- **Increased capillary permeability (k):** Circulating agents, e.g., tumor necrosis factor alpha (TNF-alpha), bradykinin, histamine, cytokines related to burn trauma, etc., increase fluid (and possibly protein) filtration resulting in edema.
- **Lymphatic obstruction/removal (lymphedema):** causes can include filarial (*W. bancrofti*: elephantitis); bacterial lymphangitis (streptococci); trauma; surgery; and tumor. Given that one function of the lymphatics is to clear interstitial proteins, lymphedema can produce a non-pitting edema because of the rise in π_{IF}.

Pulmonary Edema

Edema in the interstitium of the lung can result in grave consequences. It can interfere with gas exchange, thus causing hypoxemia and hypercapnia. A low hydrostatic pressure in pulmonary capillaries and lymphatic drainage helps to "protect" the lungs against edema.

However, similar to peripheral edema, alterations in Starling forces, capillary permeability, and/or lymphatic blockage can result in pulmonary edema. The most common causes relate to elevated capillary hydrostatic pressure and increased capillary permeability.

- **Cardiogenic (elevated P_C)** (more common)
  - Increased left atrial pressure, increases venous pressure, which in turn increases capillary pressure
  - Initially increased lymph flow reduces interstitial proteins and is protective
  - First patient sign is often orthopnea (dyspnea when supine), which can be relieved when sitting upright
  - Elevated pulmonary wedge pressure provides confirmation
  - Treatment: reduce left atrial pressure, e.g., diuretic therapy
- **Non-cardiogenic (increased permeability): adult respiratory distress syndrome (ARDS)**
- Due to direct injury of the alveolar epithelium or after a primary injury to the capillary endothelium
  - Clinical signs are severe dyspnea of rapid onset, hypoxemia, and diffuse pulmonary infiltrates leading to respiratory failure
  - Most common causes are sepsis, bacterial pneumonia, trauma, and gastric aspirations
  - Fluid accumulation as a result of the loss of epithelial integrity
  - Presence of protein-containing fluid in the alveoli inactivates surfactant causing reduced lung compliance
  - Pulmonary wedge pressure is normal or low

VOLUME MEASUREMENT OF COMPARTMENTS

To measure the volume of a body compartment, a tracer substance must be easily measured, well distributed within that compartment, and not rapidly metabolized or removed from that compartment. Use the relationship V = A/C to calculate the volume of the compartment:

$$\textbf{Volume of the compartment} = \frac{\textbf{Amount of tracer}}{\textbf{Concentration of tracer in the compartment to be measured}}$$

For example, 300 mg of a dye is injected intravenously; at equilibrium, the concentration in the blood is 0.05 mg/mL. The volume of the compartment that contained the dye is volume $= \frac{300 \text{ mg}}{0.05 \text{ mg / mL}} = 6{,}000 \text{ mL}$

This is called the volume of distribution (VOD).

Properties of the Tracer and Compartment Measured

Tracers are generally introduced into the vascular compartment, and they distribute throughout body water until they reach a barrier they cannot penetrate. The 2 major barriers encountered are **capillary membranes** and **cell membranes**. Thus, tracer characteristics for the measurement of the various compartments are as follows:

- Plasma: tracer not permeable to capillary membranes, e.g., albumin
- ECF: tracer permeable to capillary membranes but not cell membranes, e.g., inulin, mannitol, sodium, sucrose
- Total body water: tracer permeable to capillary and cell membranes, e.g., tritiated water, urea

Blood Volume versus Plasma Volume

Blood volume represents the plasma volume plus the volume of RBCs, which is usually expressed as hematocrit (fractional concentration of RBCs).

The following formula can be utilized to convert plasma volume to blood volume:

$$\textbf{Blood volume} = \frac{\textbf{plasma volume}}{\textbf{1} - \textbf{hematocrit}}$$

For example, if the hematocrit is 50% (0.50) and plasma volume = 3 L, then:

$$\textbf{Blood volume} = \frac{\textbf{3 L}}{\textbf{1} - \textbf{0.5}} = \textbf{6 L}$$

If the hematocrit is 0.5 (or 50%), the blood is half RBCs and half plasma. Therefore, blood volume is double the plasma volume.

Blood volume can be estimated by taking 7% of the body weight in kgs. For example, a 70 kg individual has an approximate blood volume of 5.0 L.

The distribution of intravenously administered fluids is as follows:

- Vascular compartment: whole blood, plasma, dextran in saline
- ECF: saline, mannitol
- Total body water: D5W–5% dextrose in water (once the glucose is metabolized, the water distributes 2/3 ICF and 1/3 ECF)

Recall Question

What is the most likely pathophysiology for cardiogenic pulmonary edema?

A. Increased pulmonary capillary permeability

B. Decreased vascular oncotic pressure

C. Increased pulmonary capillary hydrostatic pressure

D. Increased interstitial oncotic pressure

E. Lymphatic obstruction

Answer: C

PART II

Excitable Tissue

Ionic Equilibrium and Resting Membrane Potential

1

Learning Objectives

- ❑ Explain information related to overview of excitable tissue
- ❑ Interpret scenarios on ion channels
- ❑ Explain information related to equilibrium potential

EXCITABLE TISSUE

The figure below provides a basic picture of excitable cells and the relative concentration of key electrolytes inside versus outside the cell. The intracellular proteins have a negative charge. In order to understand what governs the conductance of ions as it relates to the function of excitable tissue (nerves and muscle), remember this relative difference in concentrations for these ions.

In addition, know the following key principles.

1. **Membrane potential (E_m)**: There is a separation of charge across the membrane of excitability tissue at rest. This separation of charge means there is the potential to do work and is measured in volts. Thus, E_m represents the measured value.
2. **Electrochemical gradient** indicates the combination of 2 forces: ions diffuse based upon chemical (concentration) gradients (high to low) and electrical gradients (like charges repel, opposites attract).
3. **Equilibrium potential** is the membrane potential which puts an ion in electrochemical equilibrium, i.e., the membrane potential that results in no NET diffusion of an ion. If reached, the tendency for an ion to diffuse in one direction based upon the chemical gradient is countered by the electrical force in the opposite direction. The equilibrium potential for any ion can be calculated by the Nernst equation.
4. **Conductance (g)** refers to the flow of an ion across the cell membrane. Ions move across the membrane via channels. Open/closed states of channels determine the relative permeability of the membrane to a given ion and thus the conductance. Open states create high permeability and conductance, while closed states result in low permeability and conductance.
5. **Net force (driving force)** indicates the relative "force" driving the diffusion of an ion. It is estimated by subtracting the ion's equilibrium potential from the cell's membrane potential. In short, it quantitates how far a given ion is from equilibrium at any membrane potential.

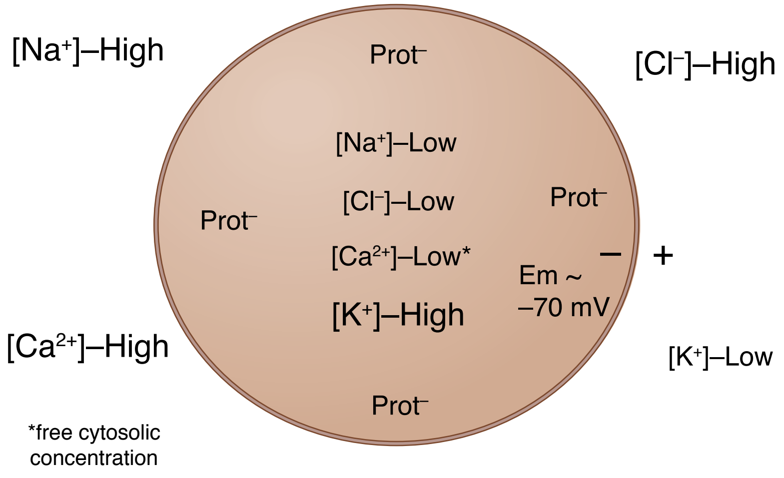


Figure II-1-1. Basic Schematic of an Excitable Cell

ION CHANNELS

Ions diffuse across the membrane via ion channels. There are 3 types:

Ungated (Leak) Ion Channel

- Always open
- Direction the ion moves depends upon electrochemical forces
- Important for determining resting membrane potential of a cell

Voltage-Gated Ion Channel

- Open/closed state is determined primarily by membrane potential (voltage)
- Change in membrane potential may open or close the channel

Ligand-Gated Ion Channel

- Channel contains a receptor
- State of the channel (open or closed) is influenced by the binding of a ligand to the receptor
- Under most circumstances, the binding of the ligand opens the channel

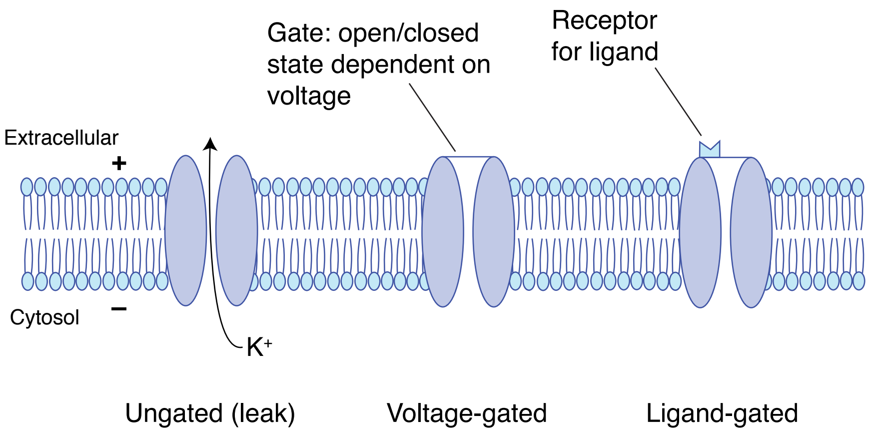


Figure II-1-2. Classes of Ion Channel

There is one **exception** to the 3 classes: the **NMDA (N-methyl-D-aspartic acid) receptor** is both voltage- and ligand-gated.

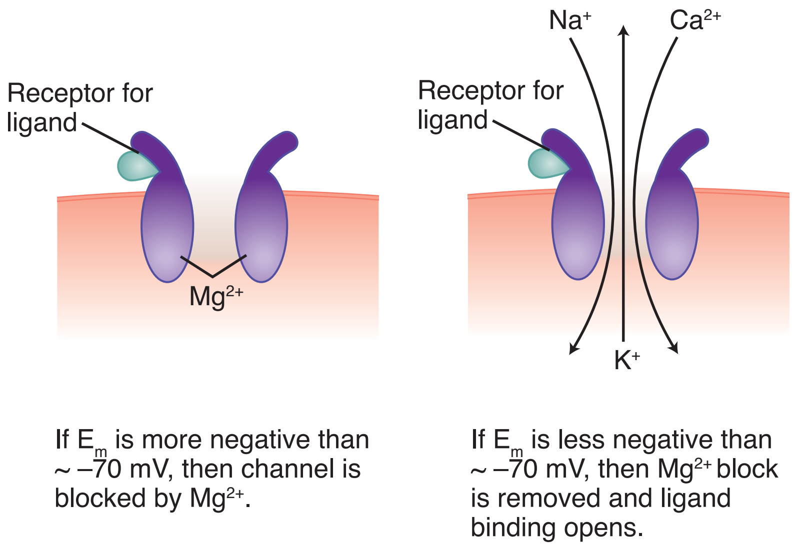


Figure II-1-3. NMDA Receptor

The pore of the NMDA receptor is blocked by Mg^{2+} if E_m is more negative than ~ –70 mV. If E_m becomes less negative than ~ –70 mV, this Mg^{2+} block is removed. Thus, the NMDA receptor exhibits characteristics of a **voltage-gated channel.**

The NMDA receptor also exhibits characteristics of a **ligand-gated channel**. Glutamate and aspartate are the endogenous ligands for the NMDA receptor. Binding of one of the ligands is **required** to open the channel.

- If E_m is more negative than ~ –70 mV, binding of the ligand does **not** open the channel (Mg^{2+} block related to voltage prevents).
- If E_m is less negative than ~ –70 mV, binding of the ligand opens the channel (even though no Mg^{2+} block at this E_m, channel will not open without ligand binding).

The NMDA receptor is a non-selective cation channel (Na^+, K^+, and Ca^{2+} flux through it). Thus, opening of this channel results in depolarization.

Although the NMDA receptor is likely involved in a variety of functions, the most important are **memory** and **pain transmission**. With respect to memory, NMDA has been shown to be involved in long-term potentiation of cells, thought to be an important component of memory formation. With respect to pain transmission, NMDA is expressed throughout the CNS and has been proven to play a pivotal role in the transmission and ultimate perception of pain.

EQUILIBRIUM POTENTIAL

Equilibrium potential is the membrane potential that puts an ion in electrochemical equilibrium. It can be calculated using the **Nernst equation**, which computes the equilibrium potential for any ion based upon the concentration gradient.

E_{X^+}: equilibrium potential

$[X^+]_o$: concentration outside (extracellular)

$[X^+]_i$: concentration inside (intracellular)

Z: value of the charge

$$E_{X^+} = \frac{60}{Z} \log_{10} \frac{[X^+]_o}{[X^+]_i}$$

Key points regarding the Nernst equation:

- The ion always diffuses in a direction that brings the E_m toward its equilibrium.
- The overall conductance of the ion is directly proportional to the net force and the permeability (determined by ion channel state) of the membrane for the ion.
- The E_m moves toward the E_X of the most permeable ion.
- The number of ions that actually move across the membrane is negligible. Thus, opening of ion channels does not alter intracellular or extracellular concentrations of ions under normal circumstances.

It is difficult to measure the intracellular concentration of the important electrolytes, so **equilibrium potential for these ions will vary**. The following are reasonable numbers to keep in mind:

E_{K+} ~ –95 mV E_{Na+} ~ +70 mV

E_{Cl-} ~ –76 mV $E_{Ca2}{}^{+}$ ~ +125 mV

Note that in **depolarization**, E_m becomes less negative (moves toward zero). In **hyperpolarization,** E_m becomes more negative (further from zero).

Resting Membrane Potential

Potassium (K^+)

There is marked variability in the resting membrane potential (rE_m) for excitable tissues, but the following generalizations are applicable.

- rE_m for nerves is ~ –70 mV while rE_m for striated muscle is ~ –90 mV.
- Excitable tissue has a considerable number of leak channels for K^+, but not for Cl^-, Na^+, or Ca^{2+}. Thus, K^+ conductance (g) is high in resting cells.
- Because of this high conductance, rE_m is altered in the following ways by changes in the extracellular concentration of K^+:
  - **Hyperkalemia depolarizes the cell.** If acute, excitability of nerves is increased (nerve is closer to threshold for an action potential) and heart arrhythmias may occur.
  - **Hypokalemia hyperpolarizes the cell.** This decreases the excitability of nerves (further from threshold) and heart arrhythmias may occur.

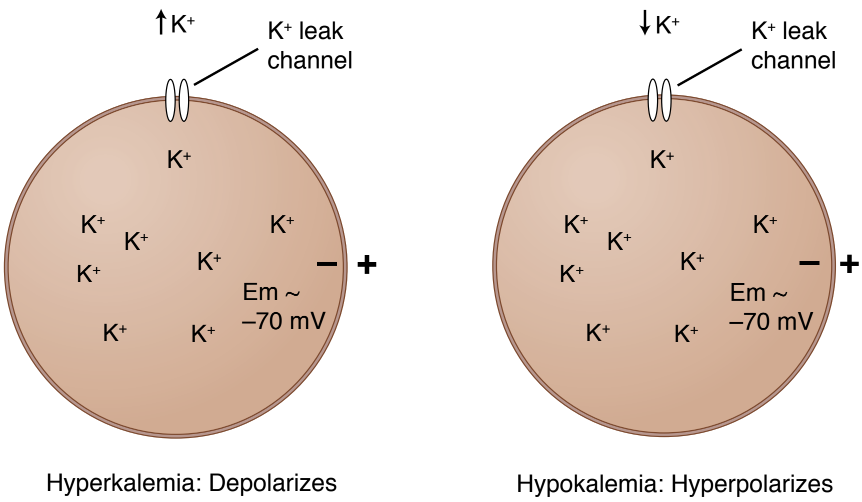


Figure II-1-4. Effect of Changes in Extracellular K^+ on Resting Membrane Potential

Altering the g for K^+ has the following effects:

- **Increasing g** causes K^+ to leave the cell, resulting in hyperpolarization of the cell. Recall that increasing g for an ion causes the E_m to move toward the equilibrium potential for that ion. Thus, the cell will move from –70 mV toward –95 mV.
- **Decreasing g** depolarizes the cell (cell moves away from K^+ equilibrium). This applies to K^+ because of its high resting g.

The Na^+/K^+ ATPase

Although the cell membrane is relatively impermeable to Na^+, it is not completely impervious to it. Thus, some Na^+ does leak into excitable cells. This Na^+ leak into the cells is counterbalanced by pumping it back out via the Na^+/K^+ ATPase. Important attributes of this pump are:

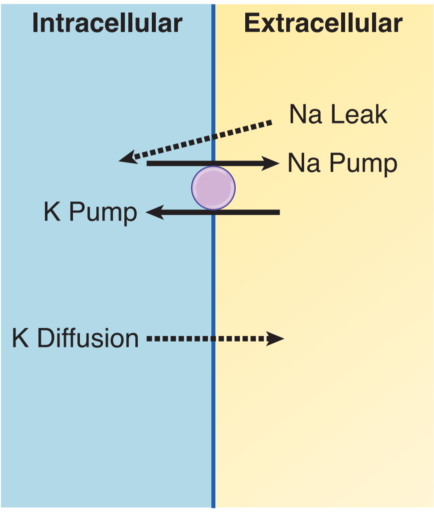


Figure II-1-5. Steady-State Resting Relationship between Ion Diffusion and Na/K-ATPase Pump

- The stoichiometry is 3 Na^+ out, 2 K^+ in. This means the pump is electrogenic because more positive charges are removed from inside the cell than are replaced. This helps maintain a negative charge inside the cell.
- Three solutes are pumped out in exchange for 2 solutes. This causes a net flux of water out of the cell. This pump is important for volume regulation of excitable tissue.

Chloride (Cl^-)

Cl^- g is low at rest. Thus, decreasing g or changing the extracellular concentration has minimal effect on rE_m.

Assuming rE_m is –70 mV, increasing Cl^- g hyperpolarizes the cell (E_m moves toward equilibrium for Cl^-, which is –76 mV). If rE_m is –80 mV or more negative, increasing Cl^- g depolarizes the cell.

Sodium (Na^+)

Na^+ g is very low at rest. Thus, decreasing g or changing the extracellular concentration has no effect on rE_m. Increasing Na^+ g depolarizes the cell (E_m moves to equilibrium for Na^+, which is +70 mV).

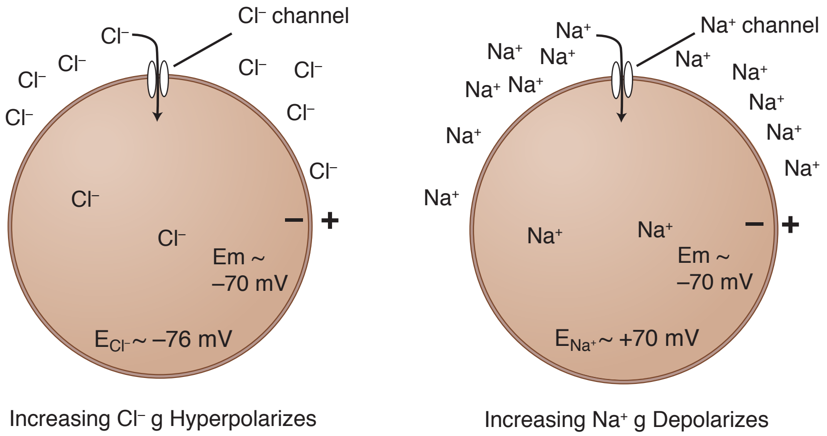


Figure II-1-6. Effect of Increased Cl^- g (left) or Na^+ g (right)

Calcium (Ca^{2+})

Ca^{2+} g is very low at rest. Thus decreasing g or changing the extracellular concentration has no effect on rE_m. Increasing Ca^{2+} g depolarizes the cell (E_m moves toward equilibrium for Ca^{2+}, which is +125 mV).

Recall Question

Which of the following is the mechanism of action behind heart arrhythmias caused by hyperkalemia?

A. Increased potassium hyperpolarizes the cell

B. Increased potassium prolongs action potential duration

C. Increased potassium increases heart rate via funny current channels

D. Increased potassium depolarizes the cell bringing excitable nerves closer to action potential

E. Increased potassium increases intracellular calcium concentration

Answer: D

The Neuron Action Potential and Synaptic Transmission

2

Learning Objectives

- ❑ Explain information related to overview of the action potential
- ❑ Solve problems concerning voltage-gated ion channels
- ❑ Demonstrate understanding of the action potential
- ❑ Use knowledge of properties of action potentials
- ❑ Answer questions about synaptic transmission
- ❑ Interpret scenarios on review and integration

ACTION POTENTIAL

The action potential is a rapid depolarization followed by a repolarization (return of membrane potential to rest). The function is:

- **Nerves**: to conduct neuronal signals
- **Muscle**: to initiate a contraction

The figure below shows the action potential from 3 types of excitable cell. Even though there are many similarities in the cell types, there are differences—most notably, the duration of the action potential.

Note the different time scales.

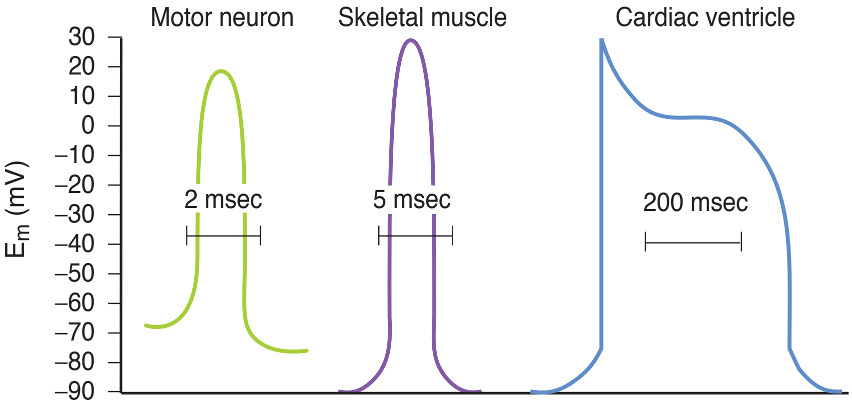


Figure II-2-1. Action Potentials from 3 Vertebrate Cell Types
(Redrawn from Flickinger, C.J., et al.: Medical Cell Biology, Philadelphia, 1979, W.B. Saunders Co.)

Note

The action potential of **nerves** is discussed in this chapter; however, since the action potential of **skeletal muscle** is virtually the same, apply the same rules. Because the **cardiac muscle** action potential has several differences, it will be discussed in the next chapter.

VOLTAGE-GATED ION CHANNELS

To understand how the action potential is generated, the ion channels involved must be discussed.

Voltage-Gated (Fast) Na^+ Channels

The opening of these channels is responsible for the rapid depolarization phase (upstroke) of the action potential. The fast Na^+ channel has 2 gates and 3 conformational states.

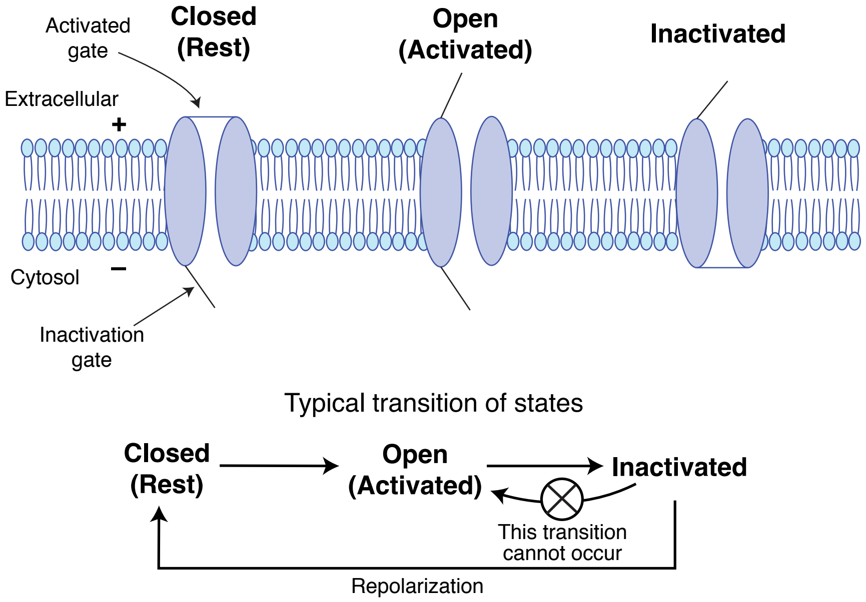


Figure II-2-2. Voltage-Gated (Fast) Na^+ Channel

- **Closed:** In the closed state, the activation gate (m-gate) is closed and the inactivation gate (h-gate) is open. Because the activation gate is closed, Na^+ conductance (g) is low.
- **Open:** Depolarization causes the channel to transition to the open state, in which both gates are open and thus Na^+ g increases. The elevated Na^+ g causes further depolarization, which in turn opens more Na^+ channels, causing further depolarization. In short, a positive-feedback cycle can be initiated if enough Na^+ channels open at or near the same time. Bear in mind, there are numerous fast Na^+ channels in every cell, and each one has its own threshold voltage for opening.
- **Inactivated:** After opening, the fast Na^+ channel typically transitions to the inactivated state. In this state, the activation gate is open and inactivation gate (h-gate) is closed. Under normal circumstances, this occurs when membrane potential becomes positive as a result of the action potential.
- Once the cell repolarizes, the fast Na^+ channel transitions back to the closed state, and is thus ready to reopen to cause another action potential.

Clinical Correlate

Hyperkalemia depolarizes neurons. Acutely, this increases excitability because the cell is closer to threshold. However, this depolarization opens some fast Na^+ channels. Over time, these channels transition into the inactivated state. Because E_m never returns to its original resting E_m (hyperkalemia keeps cell depolarized), the fast Na^+ channel is unable to transition back to the closed state and is thus "locked" in the inactivated state. This reduces the number of fast Na^+ channels available to open, resulting in the reduced neuronal excitability seen with chronic hyperkalemia.

Once an Na^+ channel inactivates, it cannot go back to the open state until it transitions to the closed state (typically when the cell repolarizes). There are some conditions in which the transition to the closed state does not occur.

Extracellular Ca^{2+} blocks fast Na^+ channels.

Voltage-Gated K^+ Channels

- Closed at resting membrane potential
- Depolarization opens, but kinetics are much slower than fast Na^+ channels
- Primary mechanism for repolarization

THE ACTION POTENTIAL

Subthreshold Stimulus

In the figure below, the blue and purple lines show changes in membrane potential (E_m) to increasing levels of stimuli, but neither result in an action potential. Thus, these are subthreshold stimuli.

- The degree of depolarization is related to the magnitude of the stimulus.
- The membrane repolarizes (returns to rest).
- It can summate, which means if another stimulus is applied before repolarization is complete, the depolarization of the second stimulus adds onto the depolarization of the first (the 2 depolarizations sum together).

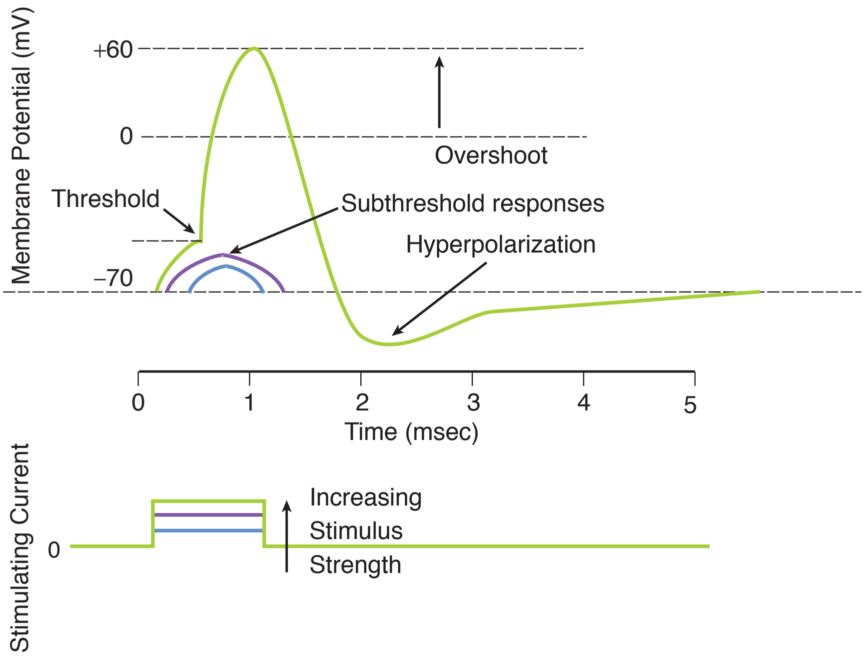


Figure II-2-3. Neuron Action Potential

Bridge to Pharmacology

Tetrodotoxin (TTX), saxitoxin (STX), and local anesthetics ("caine drugs") block fast Na^+ channels, thereby preventing an action potential.

Bridge to Pharmacology

Ciguatoxin (CTX: fish) and batrachotoxin (BTX: frogs) are toxins that block inactivation of fast Na^+ channels.

Threshold Stimulus

The green line in the figure depicts the action potential. Provided the initial stimulus is great enough to depolarize the neuron to threshold, then an action potential results. The following represents the events which occur during an action potential, which is an application of the aforementioned discussion on ion channels.

- At threshold, a critical mass of fast Na^+ channels open, resulting in further depolarization and the opening of more fast Na^+ channels.
- Because Na^+ g is high (see also Figure II-2-4), the E_m potential rapidly approaches the equilibrium potential for Na^+ (~ +70 mV)
- As membrane potential becomes positive, fast Na^+ channels begin to inactivate (see above), resulting in a rapid reduction in Na^+ conductance (see also Figure II-2-4).
- Voltage-gated K^+ channels open in response to the depolarization, but since their kinetics are much slower, the inward Na^+ current (upstroke of the action potential) dominates initially.
- K^+ g begins to rise as more channels open. As the rise in E_m approaches its peak, fast Na^+ channels are inactivating, and now the neuron has a high K^+ g and a low Na^+ g (see also Figure II-2-4).
- The high K^+ g drives E_m toward K^+ equilibrium (~ −95 mV) resulting in a rapid repolarization.
- As E_m becomes negative, K^+ channels begin to close, and K^+ g slowly returns to its original level. However, because of the slow kinetics, a period of hyperpolarization occurs.

Key Points

- The upstroke of the action potential is mediated by a Na^+ current (fast Na^+ channels).
- Although the inactivation of fast Na^+ channels participates in repolarization, the dominant factor is the high K^+ g due to the opening of voltage-gated K^+ channels.
- The action potential is all or none: Occurs if threshold is reached, doesn't occur if threshold is not reached.
- The action potential cannot summate.
- Under normal conditions, the action potential regenerates itself as it moves down the axon, thus it is propagated (magnitude is unchanged).

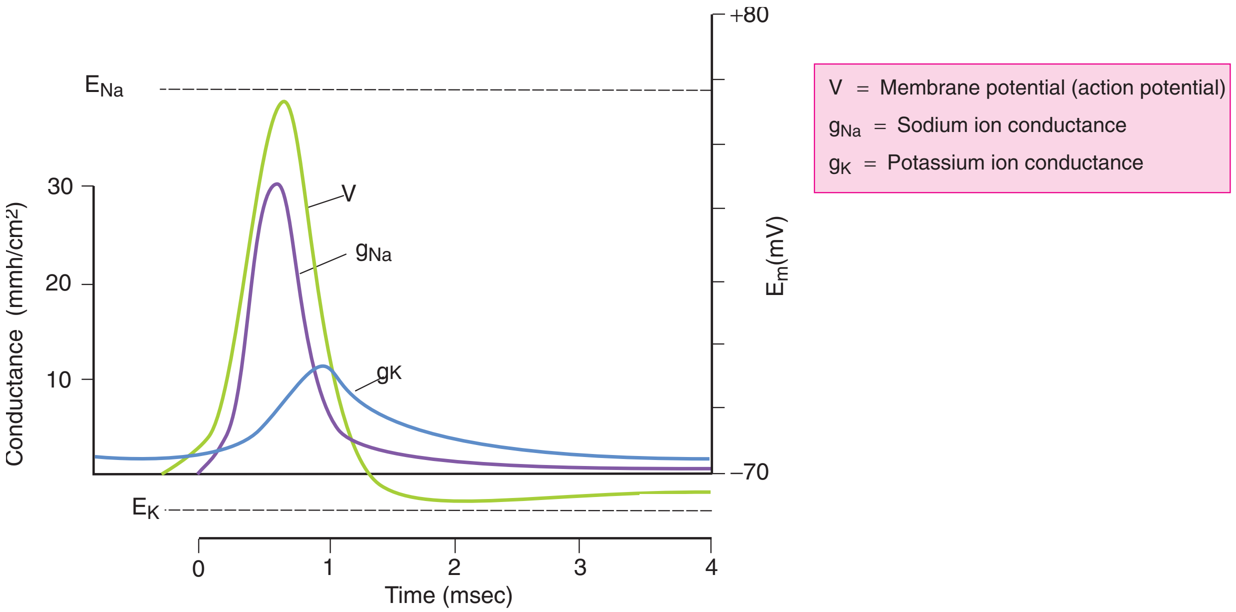


Figure II-2-4. Axon Action Potential and Changes in Conductance

PROPERTIES OF ACTION POTENTIALS

Refractory Periods

The **absolute refractory period** is the period during which no matter how strong the stimulus, it cannot induce a second action potential. The mechanism underlying this is the fact that during this time, most fast Na^+ channels are either open or in the inactivated state. The approximate duration of the absolute refractory period is seen below; the length of this period determines the maximum frequency of action potentials.

The **relative refractory period** is that period during which a greater than threshold stimulus is required to induce a second action potential. The mechanism for this is the elevated K^+ g.

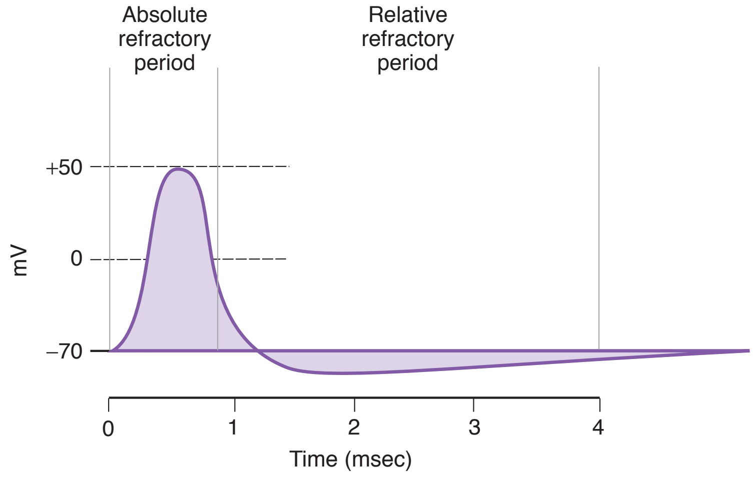


Figure II-2-5. Refractory Periods

Bridge to Pathology

Multiple sclerosis (MS) and Guillain-Barre syndrome (GBS) are demyelinating diseases. Loss of myelin results in current leakage across the membrane. The magnitude of current reaching the cluster of fast Na^+ channels is unable to cause threshold depolarization, resulting in a conduction block. MS preferentially demyelinates neurons in the CNS, while GBS acts on peripheral neurons.

Note

The basics of neurotransmitter release described in this section are applicable to synaptic transmission for all synapses.

Conduction Velocity of the Action Potential

There are 2 primary factors influencing conduction velocity in nerves:

- **Cell diameter**: The greater the cell diameter, the greater the conduction velocity. A greater cross-sectional surface area reduces the internal electrical resistance.
- **Myelination**: Myelin provides a greater electrical resistance across the cell membrane, thereby reducing current "leak" through the membrane. The myelination is interrupted at the nodes of Ranvier where fast Na^+ channels cluster. Thus, the action potential appears to "bounce" from node to node with minimal decrement and greater speed (saltatory conduction).

SYNAPTIC TRANSMISSION

Neuromuscular Junction

The synapse between the axons of an alpha-motor neuron and a skeletal muscle fiber is called the neuromuscular junction (NMJ). The terminals of alpha-motor neurons contain acetylcholine (Ach), thus the synaptic transmission at the neuromuscular junction is one example of cholinergic transmission.

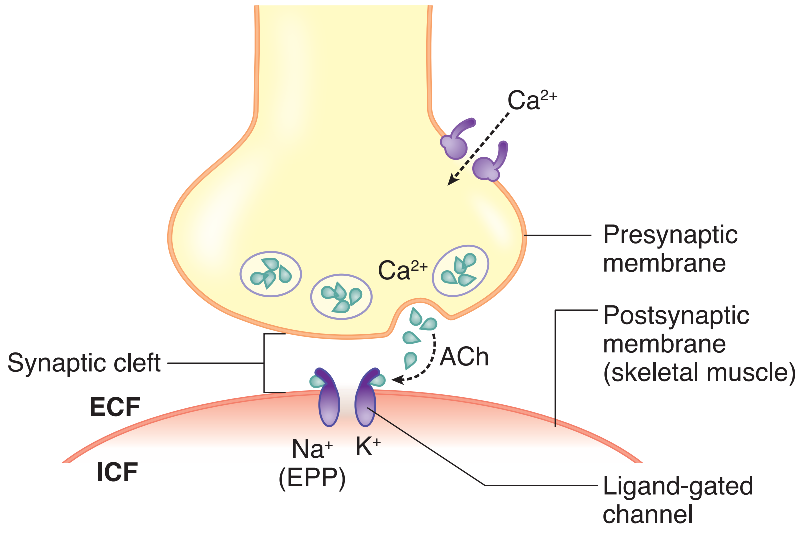


Figure II-2-6. Neuromuscular Transmission and Nicotinic Synapses

Sequence of events

1. The action potential travelling down the motor neuron depolarizes the presynaptic membrane.
2. This depolarization opens voltage-gated Ca^{2+} channels in the presynaptic membrane, resulting in Ca^{2+} influx into the presynaptic terminal.

3. The rise in Ca^{2+} causes synaptic vesicles to release their contents, in this case, Ach. The amount of neurotransmitter release is **directly related** to the rise in cytosolic Ca^{2+}, i.e., the more Ca^{2+} that enters, the more neurotransmitter released.
4. Ach binds to a nicotinic receptor located on the muscle membrane (N_M receptor). The N_M receptor is a non-selective monovalent cation channel (both Na^+ and K^+ can traverse). Given that Na^+ has a much greater net force (see Chapter 1 of this section), depolarization occurs. This depolarization is called an end-plate potential (EPP). The magnitude of the EPP is directly related to the amount of Ach released.
5. The resulting depolarization opens fast Na^+ channels on the muscle membrane (sarcolemma) causing an action potential in the sarcolemma. Under normal circumstances, an action potential in the motor neuron releases enough Ach to cause an EPP that is at least threshold for the action potential in the skeletal muscle cell. In other words, there is a one-to-one relationship between an action potential in the motor neuron and an action potential in the skeletal muscle cell.
6. The actions of Ach are terminated by acetylcholinesterase (AchE), an enzyme located on the postsynaptic membrane that breaks down Ach into choline and acetate. Choline is taken back into the presynaptic terminal (reuptake), hence providing substrate for re-synthesis of Ach.

Synapses Between Neurons

The figure below illustrates synaptic junctions between neurons. In general, the synaptic potentials produced are excitatory or inhibitory, and they are produced by **ligand-gated ion channels**.

- Synapses are located on the cell body and dendrites.
- The currents produced at these synapses travel along the dendritic and cell body membranes.
- The axon hillock–initial segment region has a high density of fast Na^+ channels and is the origin for the action potential of the axon.
- The closer the synapse is to this region, the greater its influence in determining whether an action potential is generated.
- If the sum of all the inputs reaches threshold, an action potential is generated and conducted along the axon to the nerve terminals.

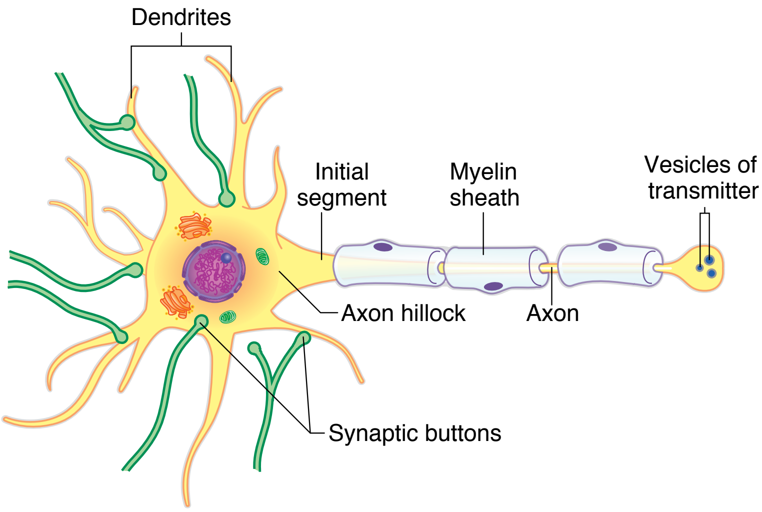


Figure II-2-7. Synapse Transmission between Neurons

Excitatory postsynaptic potential (EPSP) is excitatory if it increases the excitability of the postsynaptic neuron, i.e., it is more likely to fire an action potential. It is primarily the result of increased Na^+ g. It is similar to the EPP found at the neuromuscular junction.

- Important receptors that produce:
  - Nicotinic: endogenous ligand is Ach and include N_M and N_N.
  - Non-NMDA (N-methyl-D-aspartic acid): endogenous ligands are glutamate and aspartate (excitatory amino acid transmitters), and Na^+ g is increased when they bind
  - NMDA: endogenous ligands are the excitatory amino acids and it is a non-selective cation channel (discussed in the preceding chapter).

Inhibitory postsynaptic potential (IPSP) is inhibitory if it decreases the excitability of the postsynaptic neuron, i.e., it is less likely to fire an action potential. It is primarily the result of increased Cl^- g.

- Important receptors that produce:
  - $GABA_{A\&C}$: endogenous ligand is GABA (gamma-aminobutyric acid)
  - Glycine: endogenous ligand is glycine

Electrical Synapses

In contrast to chemical synaptic transmission, in electrical synapses there is a direct flow of current from cell to cell. The cell-to-cell communication occurs via gap junctions; because the cells are electrically coupled, there is no synaptic delay. Cardiac and single-unit smooth muscle cells have these electrical synapses.

Peripheral Nervous System

Motor

Alpha-motor neurons release Ach, which binds to the N_M (nicotinic muscle) receptor. These are large, well-myelinated neurons, i.e., they exhibit fast conduction.

Parasympathetic nervous system

Preganglionic neurons release Ach, which binds to N_N (nicotinic neuronal) receptor. Postganglionic fibers release Ach, which binds to muscarinic receptor (G-protein coupled).

Sympathetic nervous system

Preganglionic neurons release Ach, which binds to N_N receptor.

Postganglionic neurons (most) release norepinephrine (NE), which binds to alpha and beta (β-1 & β-3) receptors (G-protein coupled).

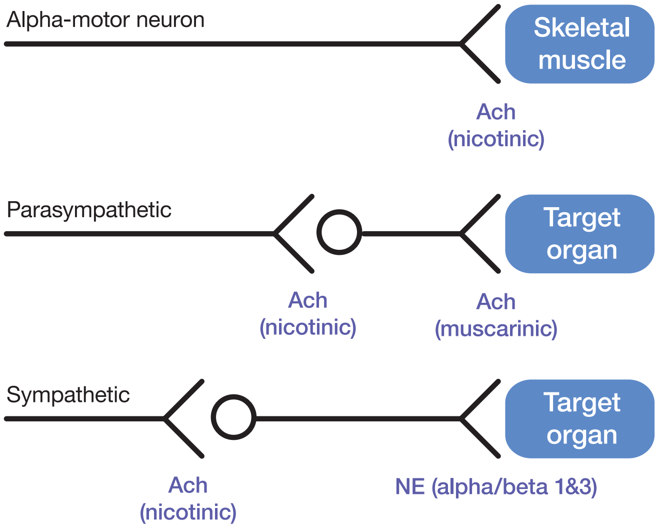


Figure II-2-8. Peripheral Nervous System

REVIEW AND INTEGRATION

In this section, we review much of the preceding information and add in applicable new information as it pertains to clinical signs indicative of alterations in the normal physiological function just discussed. These are clinical signs intended to help further reinforce the important physiology and thus aid the student in recognizing possible causes of these clinical signs.

This is not intended to fully represent all the specific signs/symptoms related to each and every condition indicated.

Decreased Neuronal Excitability/Conduction

Clinical signs could include: weakness; ataxia; hyporeflexia; paralysis; sensory deficit. Possible causes include the following:

Table II-2-1.

Ion Disturbances	Loss of Neurons/ Demyelination	Toxins/Drugs	NMJ
Hypokalemia	Guillian-Barre	Local anesthetics ("caine" drugs)	Depolarizing N_M blockers
Chronic hyperkalemia	ALS (amyotrophic lateral sclerosis)	TTX	Non-depolarizing N_M blockers
Hypercalcemia	Aging	STX	Lambert-Eaton
			Myasthenia gravis
			Botulinum

Increased Neuronal Excitability/Conduction

Clinical signs could include: hyperreflexia, spasms, muscle fasciculations, tetany, tremors, paresthesias, and convulsions. Possible causes include the following:

Table II-2-2.

Ion Disturbances	Loss of Neurons/ Demyelination	Toxins/Drugs	NMJ
Acute hyperkalemia	Multiple sclerosis	CTX	AchE inhibitors
Hypocalcemia		BTX	Latrotoxin

Recall Question

Which of the following represents the pathologic alteration causing myasthenia gravis?

A. Autoimmune with antibodies that block postsynaptic Nm receptors

B. Antibodies blocking the presynaptic voltage-gated Ca2+ channels

C. Opening of presynaptic Ca2+ channels resulting in excess ACh release

D. Toxins that block inactivation of fast Na+ channels

E. Demyelination of Schwann cells

Answer: A

Bridge to Pharmacology

Botulinum toxin is a protease that destroys proteins needed for the fusion and release of synaptic vesicles. This toxin targets cholinergic neurons, resulting in flaccid paralysis.

Bridge to Pharmacology

Latrotoxin, the venom from the black-widow spider, opens presynaptic Ca^{2+} channels, resulting in excess Ach release.

Bridge to Pharmacology

Many pesticides, as well as some therapeutic agents, block AchE, resulting in the prolonged action of Ach in cholinergic synapses.

Bridge to Pharmacology

A variety of compounds can block N_M receptors (non-depolarizing neuromuscular blockers), while succinylcholine binds to this receptor causing the channel to remain open (depolarizing neuromuscular blocker).

Bridge to Pathology

Two important pathologies related to neuromuscular junctions are **myasthenia gravis** and **Lambert-Eaton** syndrome. The most common form of myasthenia gravis is an autoimmune condition in which antibodies are created that block the N_M receptor. Lambert-Eaton is also an autoimmune condition, but the antibodies block the presynaptic voltage-gated Ca^{2+} channels.

Electrical Activity of the Heart 3

Learning Objectives

- ❏ Use knowledge of properties of cardiac tissue
- ❏ Answer questions about cardiac action potentials
- ❏ Use knowledge of control of nodal excitability
- ❏ Answer questions about electrocardiology
- ❏ Explain information related to arrhythmias/ECG alterations

PROPERTIES OF CARDIAC TISSUE

Cells within the heart are specialized for different functional roles. In general, these specializations are for automaticity, conduction, and/or contraction.

Automaticity

Cardiac cells initiate action potentials spontaneously. Further, the cells are electrically coupled via gap junctions. Thus, when a cell fires an action potential, it typically sweeps throughout the heart. Although all cardiac tissue shows spontaneous depolarization, only the following 3 are germane.

- **Sinoatrial (SA) node cells** are specialized for automaticity. They spontaneously depolarize to threshold and have the highest intrinsic rhythm (rate), making them the pacemaker in the normal heart. Their intrinsic rate is ~100/min.
- **Atrioventricular (AV) node cells** have the second highest intrinsic rhythm (40-60/min). Often, these cells become the pacemaker if SA node cells are damaged.
- Although not "specialized" for automaticity per se, **Purkinje cells** do exhibit spontaneous depolarizations with a rate of ~35/min.

Conduction

All cardiac tissue conducts electrical impulses, but the following are particularly specialized for this function.

- AV node: These cells are specialized for slow conduction. They have small diameter fibers, a low density of gap junctions, and the rate of depolarization (phase 0, see below) is slow in comparison to tissue that conducts fast.

- Purkinje cells: These cells are specialized for rapid conduction. Their diameter is large, they express many gap junctions, and the rate of depolarization (phase 0, see below) is rapid. These cells constitute the HIS-Purkinje system of the ventricles.

Contraction

Although myocytes have a spontaneous depolarization and they conduct electrical impulses, they contain the protein machinery to contract.

Conduction Pathway

Because cells are electrically coupled via gap junctions, excitation to threshold of one cell typically results in the spread of this action potential throughout the heart. In the normal heart, the SA node is the pacemaker because it has the highest intrinsic rhythm.

The normal conduction pathway for the heart is as follows:

atrial muscle
↗
SA node → internodal fibers → AV node (delay) → Purkinje fibers → ventricular muscle

CARDIAC ACTION POTENTIALS

Resting Membrane Potential (Non-Nodal Cells)

Potassium conductance is high in resting ventricular or atrial myocytes. This is also true for Purkinje cells. Because of this, resting membrane potential is close to K^+ equilibrium potential. This high-resting K^+ conductance is the result to 2 types of channels.

Ungated potassium channels

Always open, and unless the membrane potential reaches the potassium equilibrium potential (~ –95 mV), a potassium flux (efflux) is maintained through these channels.

Inward K^+ rectifying channels (IK_1)

- Voltage-gated channels that are open at rest.
- Depolarization closes.
- They open again as the membrane begins to repolarize.

Action Potential (Non-Nodal Cells)

Understanding the ionic basis of cardiac action potentials is important for understanding both cardiac physiology and the electrocardiogram (ECG), which is a recording of the currents produced by these ionic changes. In addition, antiarrhythmic drugs exert their effects by binding to the channels that produce these ionic currents.

In this section, we review the various phases of the action potentials that occur in myocytes and Purkinje cells. Action potentials generated by nodal cells (SA and AV) are discussed later. Although there are slight differences in the action potentials generated by atrial and ventricular myocytes, as well as Purkinje cells, these differences are not included here.

Furthermore, remember that cardiac cells are electrically coupled by gap junctions. Thus, when a cell fires an action potential, it spreads and is conducted by neighboring cells.

The figure below shows the labeled phases of the action potential from a ventricular myocyte and the predominant ionic currents related to the various phases.

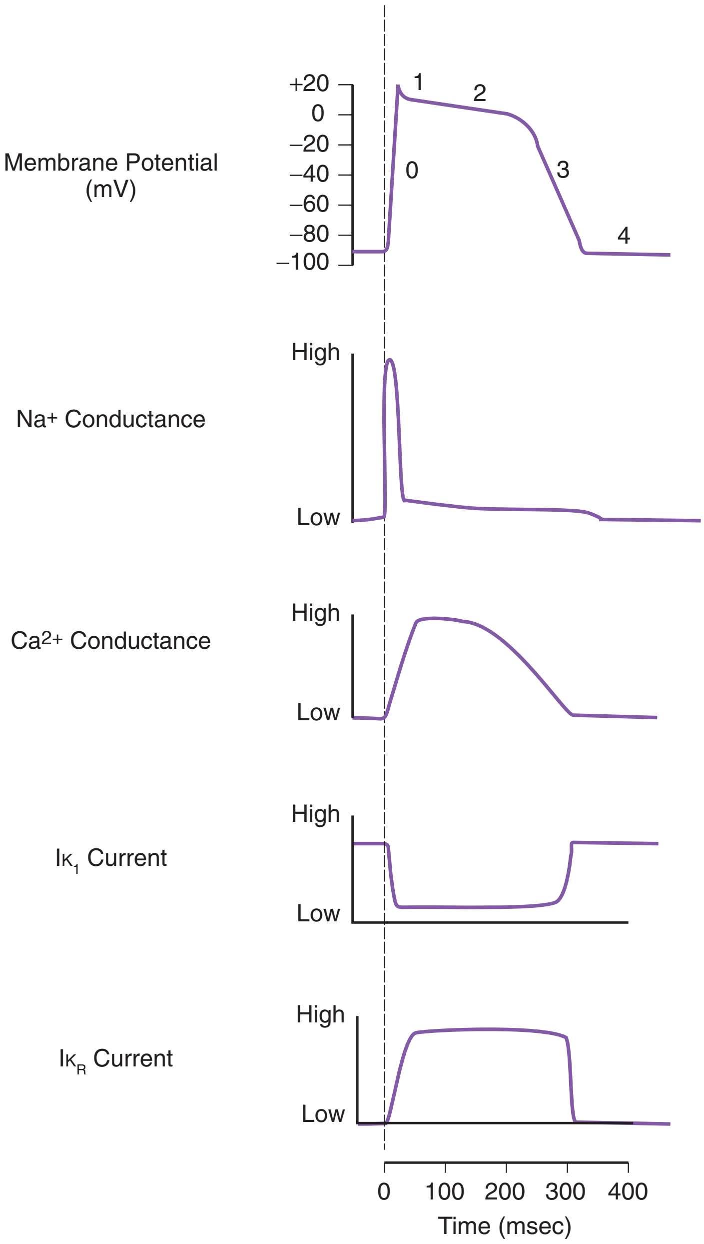


Figure II-3-1. Action Potential in a Ventricular Myocyte

Bridge to Pathology

Note that Na^+ conductance during phase 2 is still slightly elevated. Some of these channels are very slow to inactivate and data suggest that genetic alterations can result in a significant Na^+ current during phase 2. This Na^+ current delays repolarization, resulting in a prolonged QT. This genetic alteration appears to play a role in congenital long QT syndrome.

A prolonged QT interval can cause a form of ventricular tachycardia known as **torsade de pointes**. Other factors can increase the QT interval, thus possibly producing torsade de pointes.

Bridge to Pharmacology

Class I antiarrhythmic agents block fast Na^+ channels, resulting in a change in phase 0. Blocking these channels reduces conduction velocity, an action that can be beneficial, e.g., use of lidocaine to reduce conduction and stabilize the heart when the tissue becomes ischemic.

Bridge to Pharmacology

Class III antiarrhythmic drugs block K^+ channels. This delays repolarization, resulting in a long QT interval.

Phase 0

- Upstroke of the action potential
- Similar to nerve and skeletal muscle, mediated by the opening of voltage-gated, fast Na^+ channels (note high Na^+ conductance)
- Conduction velocity is directly related to rate of change in potential (slope). Stimulation of β-1 receptors, e.g., epinephrine and norepinephrine, increases the slope and thus increases conduction velocity.
- Creates the QRS complex of the ECG

Phase 1

- Slight repolarization mediated by a transient potassium current
- Sodium channels transition to the inactivated state (note reduction in Na^+ conductance).

Phase 2 (plateau)

- Depolarization opens voltage-gated Ca^{2+} channels (primarily L-type) and voltage-gated K^+ channels (IK_R current being one example).
- The inward Ca^{2+} current offset by the outward K^+ current results in little change in membrane potential (plateau).
- The influx of Ca^{2+} triggers the release of Ca^{2+} from the SR (Ca^{2+} induced Ca^{2+} release), resulting in cross-bridge cycling and muscle contraction (see next chapter).
- Creates the ST segment of the ECG
- The long duration of the action potential prevents tetany in cardiac muscle (see next chapter).

Phase 3

- Repolarization phase
- L-type channels begin closing, but rectifying K^+ currents (IK_R current being one example) still exist, resulting in repolarization.
- IK_1 channels reopen and aid in repolarization.
- Creates the T wave of the EKG

Phase 4

- Resting membrane potential
- Fast Na^+, L-type Ca^{2+}, and rectifying K^+ channels (IK_R) close, but IK_1 channels remain open.

Action Potential (Nodal Cells)

Nodal tissue (SA and AV) lacks fast Na^+ channels. Thus, the upstroke of the action potential is mediated by a Ca^{2+} current rather than an Na^+ current. In addition, note that phases 1 and 2 are absent.

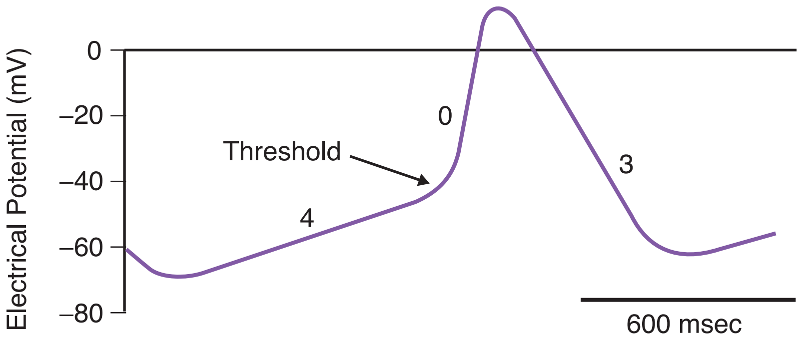


Figure II-3-2. SA Nodal (Pacemaker) Action Potential

Phase 4

- Resting membrane potential
- Given this tissue is specialized for automaticity (see above), these cells show a spontaneous depolarization at rest. This spontaneous depolarization is referred to as the "pacemaker" potential and results from:
  - **Inward Ca^{2+} current:** Primarily related to T-type Ca^{2+} channels. These differ from the L-type in that they open at a more negative membrane potential (~ -70 mV).
  - **Inward Na^+ current:** This inward Na^+ current is referred to as the "funny" current (I_f) and the channel involved is a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel. HCN are non-selective monovalent cation channels and thus conduct both Na^+ and K^+. However, opening of these channels evokes a sodium-mediated depolarization (similar to nicotinic receptors, see previous chapter). These channels open when the membrane repolarizes (negative membrane potential), and they close in response to the depolarization of the action potential.
  - **Outward K^+ current:** There is a reduced outward K^+ current as the cell repolarizes after the action potential. Reducing this current helps to produce the pacemaker potential.

Phase 0

- Upstroke of the action potential
- Mediated by opening of L-type (primarily) Ca^{2+} channels
- Note the time scale: the slope of phase 0 is not steep in nodal tissue like it is in ventricular myocytes or the upstroke of the action potential in nerves. This is part of the reason conduction velocity is slow in the AV node.

Phase 3

- Repolarization phase
- Mediated by voltage-gated K^+ channels

CONTROL OF NODAL EXCITABILITY

Catecholamines

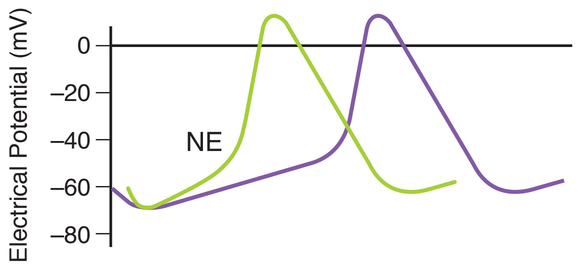


Figure II-3-3. Sympathetic Effects on SA Nodal Cells

- Norepinephrine (NE) from postganglionic sympathetic nerve terminals and circulating epinephrine (Epi)
- β-1 receptors; Gs—cAMP; stimulates opening of HCN and Ca^{2+} channels
- Increased slope of pacemaker potential (gets to threshold sooner)
- Functional effect
  - Positive chronotropy (SA node): increased HR
  - Positive dromotropy (AV node): increased conduction velocity through the AV node

Bridge to Pharmacology

Class II antiarrhythmics are the beta-blockers, while class IV antiarrhythmics are the Ca^{2+} channel blocks. These drugs reduce automaticity and conduction through the AV node and can be very efficacious in tachyarrhythmias.

Bridge to Pharmacology

Ivabradine blocks the funny current in the SA node, thereby reducing HR. It has the following uses:

- For systolic heart failure when beta-blockers fail to reduce HR sufficiently
- For idiopathic sinus tachycardia

Parasympathetic

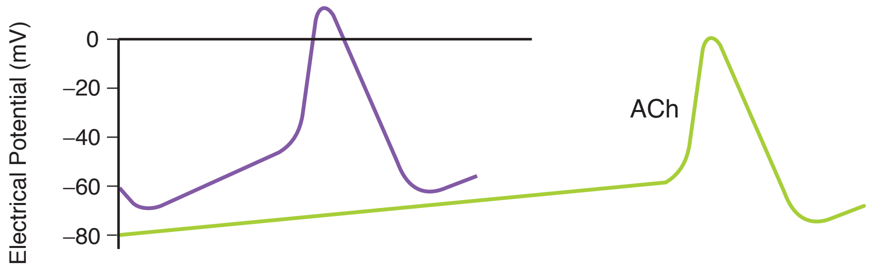


Figure II-3-4. Parasympathetic Effects on SA Nodal Cells

- Ach released from post-ganglionic fibers
- M_2 receptor; Gi-Go; Opens K^+ channels and inhibits cAMP
- Hyperpolarizes; reduced slope of pacemaker potential
- Functional effect
  - Negative chronotropy (SA node): Decreased HR
  - Negative dromotropy (AV node): Decreased conduction velocity through the AV node

ELECTROCARDIOLOGY

Electrocardiogram

The normal pattern of an electrocardiogram (EKG or ECG) is demonstrated below.

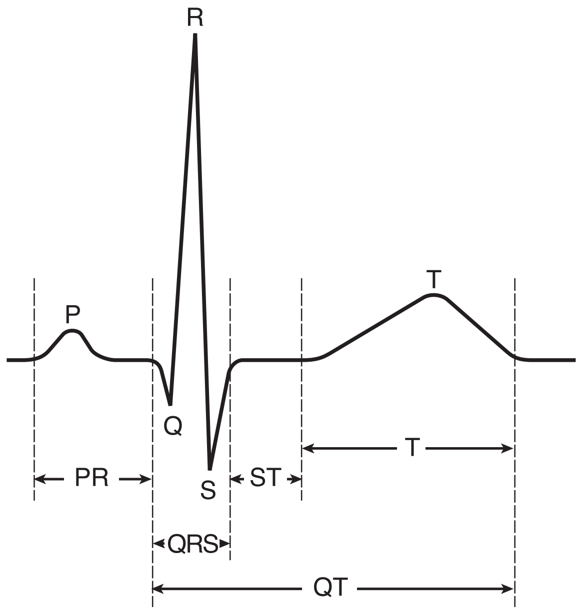


Figure II-3-5. Normal Pattern of an ECG

P wave: atrial depolarization

QRS complex: ventricular depolarization (40–100 msec)

R wave: first upward deflection after the P wave

S wave: first downward deflection after an R wave

T wave: ventricular repolarization

PR interval: start of the P wave to start of the QRS complex (120–200 msec); mostly due to conduction delay in the AV node

QT interval: start of the QRS complex to the end of the T wave; represents duration of the action potential

ST segment: ventricles are depolarized during this segment; roughly corresponds to the plateau phase of the action potential

J point: end of the S wave; represents isoelectric point

The height of waves is directly related to (a) mass of tissue, (b) rate of change in potential, and (c) orientation of the lead to the direction of current flow.

The alignment of the cardiac action potential and the ECG recording are further illustrated below.

- Phase 0 produces the QRS complex.
- Phase 3 produces the T wave.
- The ST segment occurs during phase 2.
- The QT interval represents the duration of the action potential and this interval is inversely related to heart rate. For example, stimulation of sympathetics to the heart increases heart rate and reduces the duration of the action potential, thus decreasing the QT interval.

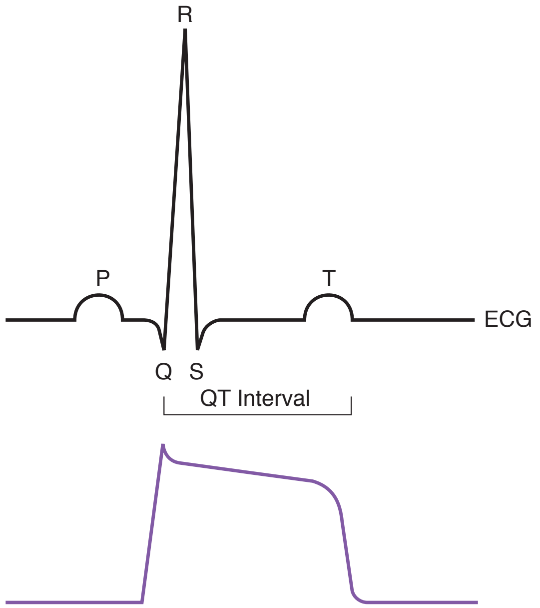


Figure II-3-6. Ventricular Action Potential vs ECG

Standard Conventions

The figure below shows a normal ECG trace from a single lead. The ECG measures volts (y-axis) per unit time (x-axis) and the scales are standardized. Note the heavier (darker) lines both horizontally and vertically. These represent “big” boxes, each of which is further subdivided into 5 “small” boxes.

- **y-axis (volts): one big box = 0.5 mV**
  - Because there are 7 big boxes above the bottom line, the total height is 3.5 mV.
- **x-axis (time): one big box = 0.2 sec (200 msec)**
  - Because there are 5 subdivisions within each big box, each small box is 0.04 sec (40 msec). Here, 5 big boxes equal 1 second.

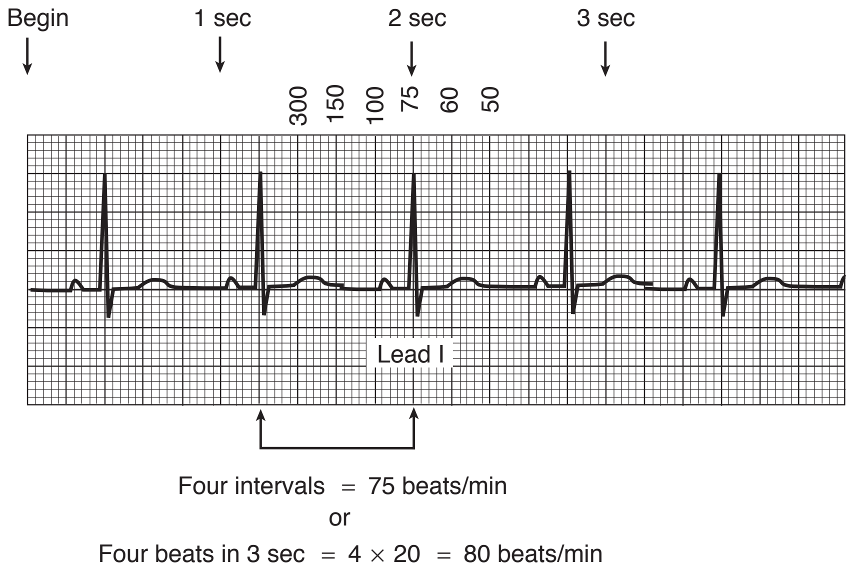


Figure II-3-7. Estimation of Heart Rate

Reading an ECG

The ECG is a powerful clinical tool, and it takes years of training to become fully competent in detecting the many abnormalities it can detect. While a detailed explanation is beyond the scope of this book, there are some arrhythmias and alterations one should be able to recognize early in medical training.

Use the following step-wise approach to help you detect alterations in the ECG.

Step 1: rate and rhythm

If provided, use the rhythm strip (lead II) that typically runs the length of the recording and is located on the bottom of the printout. We will use a single trace illustrated above.

- **Rhythm:** Qualitatively look at the trace and determine if there is a steady rhythm. This means the R waves occur regularly, i.e., the space between each is approximately the same. If so then there is a steady rhythm; if not then an unsteady rhythm.
- **Rate:** It is typically not necessary to determine the exact heart rate (HR); simply determine if it is within the normal range (60–100/min). The simplest way to do this is to find an R wave that is on a heavy (darker) vertical line, and note where the next R wave occurs with respect to the following count of subsequent heavy vertical lines:

  1 = 300 beats/min
  2 = 150
  3 = 100
  4 = 75
  5 = 60
  6 = 50

For example, if the subsequent R wave occurs at the second heavy line from the first R wave, then HR is 150 beats/min. If it occurs at the third heavy line from the first R wave, then HR is 100 beats/min, and so on. In the figure above, it occurs at the fourth heavy line, thus HR is 75 beats/min for this ECG.

If the subsequent R wave occurs between heavy lines, then the HR is between the values denoted for those lines. Even though it won't be a precise number, one can ascertain whether it is above or below the normal range.

Step 2: Waves

Qualitatively examine the trace for the presence of P, QRS, and T. Can they be seen and do they look somewhat "normal"?

Step 3: PR interval

Find the PR interval and determine if it is in the normal range (120–200 msecs). This normal range translates into 3-5 small boxes. Look at several cycles to see if the PR interval is consistent.

Step 4: Estimate the mean electrical axis

The mean electrical axis (MEA) indicates the net direction (vector) of current flow during ventricular depolarization. Each lead can be represented by an angle.

Although the MEA axis can be determined very precisely, it is not important to do so at this stage. Instead, we will define what quadrant (quadrant method) the MEA falls in using a very simplified approach.

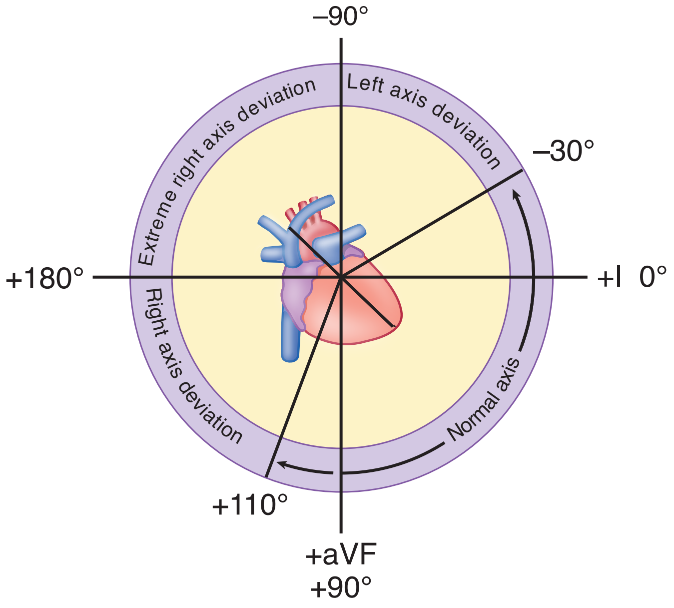


Figure II-3-8. Axis Ranges

Quadrant method

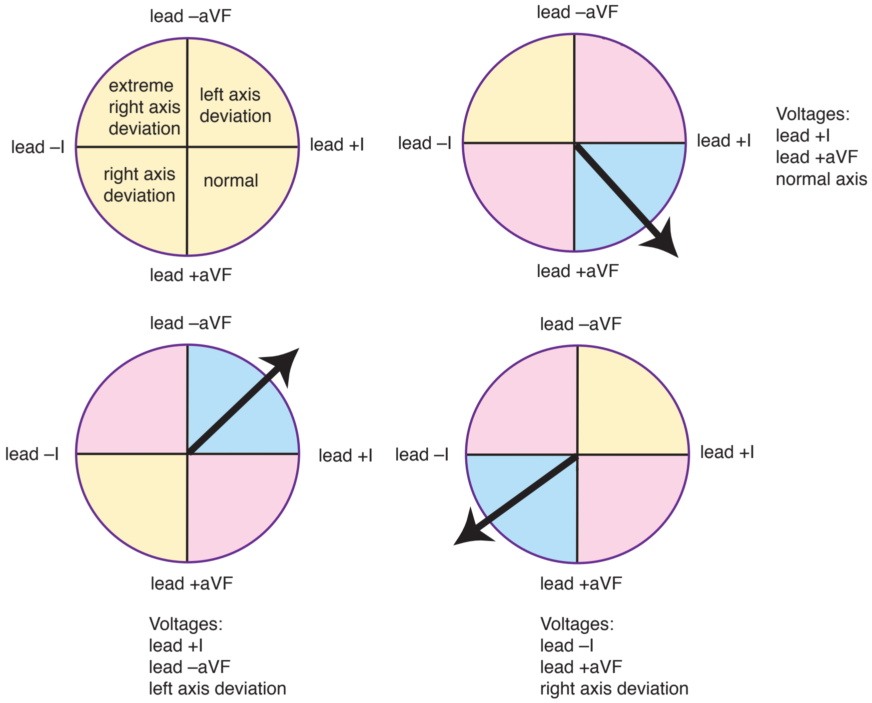


Figure II-3-9. Quadrant Method

- Determine the net QRS deflection (upward minus downward) in lead I and aVF. Using these 2 leads allows us to partition the mathematical grid into 4 basic quadrants (upper left panel of Figure II-3-9).
- If the net deflections for leads I and aVF are positive, then the MEA is between 0° and 90°, and is thus normal (upper right panel of figure above). Note: The normal range for MEA is –30° and +110°. Even though the quadrant method is not precise, it is close enough at this juncture.
- If the net deflection is positive in lead I and negative in aVF, then the MEA is between 0° and –90°, and there is a left axis deviation (lower left panel of figure above).
  - Causes of left axis deviation are:
    - Left heart enlargement, either left ventricular hypertrophy or dilation
    - Conduction defects in the left ventricle, except in the posterior bundle branch
    - Acute MI on right side tends to shift axis left unless right ventricle dilates

- If the net deflection is negative in lead I and positive in aVF, then the MEA is between 90° and 180°, and there is a right axis deviation (lower right panel of figure above.
  - Causes of right axis deviation are:
    - Right heart enlargement, hypertrophy, or dilation
    - Conduction defects of right ventricle or the posterior left bundle branch
    - Acute MI on left side tends to shift axis right unless left ventricle dilates

Recall Question

Which of the following corresponds to phase 2 of the non-nodal action potential?

A. Upstroke of the action potential creating the QRS complex of the EKG

B. Sodium channels transition to the inactivated state

C. Resting membrane potential

D. Repolarization phase creating the T wave of the EKG

E. Inward Ca2+ current offset by the outward K+ current resulting in little change in membrane potential

Answer: E

ARRHYTHMIAS/ECG ALTERATIONS

A detailed description of the various arrhythmias is beyond the scope of this book, but there are several that should be recognizable to you for the exam.

Heart Block

First-Degree

Long PR interval (>200 msec; one big box). Slowed conduction through the AV node. Rate and rhythm are typically normal

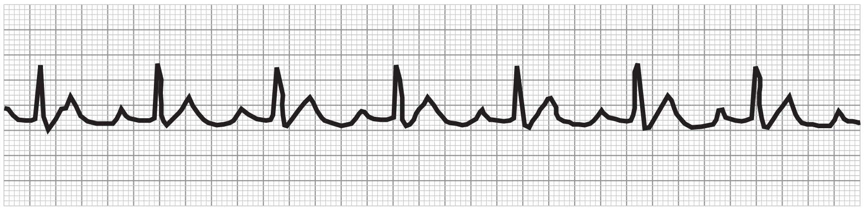


Figure II-3-10. First-Degree Heart Block

Second-Degree

Every QRS complex is preceded by a P wave, but not every P wave is followed by a QRS complex. Some impulses are not transmitted through the AV node. There are 2 types:

- **Mobitz type I (Wenckebach):** Progressive prolongation of PR interval until a ventricular beat is missed and then the cycle begins again. This arrhythmia will have an unsteady rhythm.
- **Mobitz type II:** PR interval is consistent, i.e., it doesn't lengthen and this separates it from Wenckebach. The rhythm can be steady or unsteady depending upon block ratio (P to QRS ratio: 2:1, 3:1, 3:2, etc.).

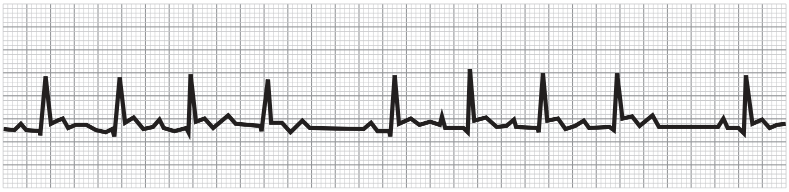


Figure II-3-11. Second-Degree Heart Block (Mobitz Type I)

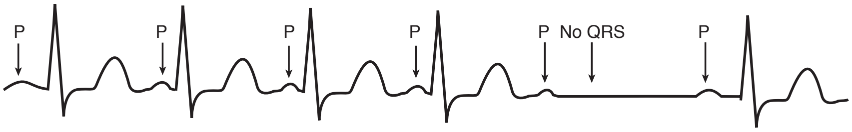


Figure II-3-12. Second-Degree Heart Block (Mobitz Type II)

Third-Degree (Complete)

There is complete dissociation of P waves and QRS complexes. Impulses are not transmitted through the AV node. Steady rhythm (usually) and very slow ventricular HR (usually); no consistent PR interval because impulses are not transmitted through the AV node; rate for P waves is different than rate for R waves.

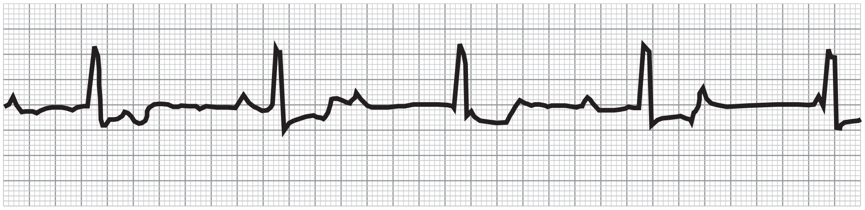


Figure II-3-13. Complete Heart Block

Atrial Flutter

Very fast atrial rate (>280 beats/min)

- Although fast, atrial conduction is still intact and coordinated.
- Characteristics: "saw-tooth" appearance of waves between QRS complexes; no discernible T waves; rhythm typically steady

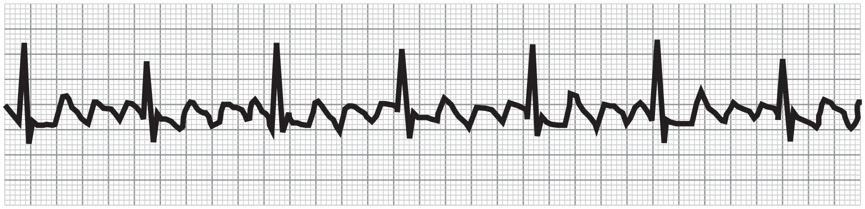


Figure II-3-14. Atrial Flutter

Atrial Fibrillation

Uncoordinated atrial conduction

- Lack of a coordinated conduction results in no atrial contraction
- Characteristics: unsteady rhythm (usually) and no discernible P waves

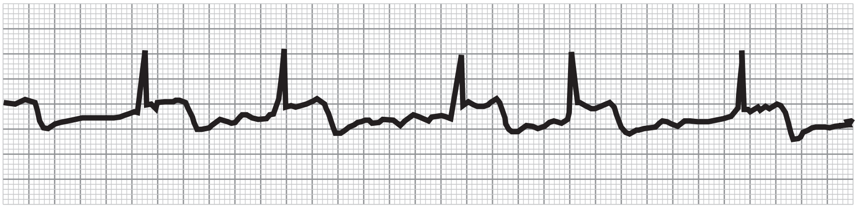


Figure II-3-15. Atrial Fibrillation

Wolff-Parkinson-White Syndrome

Accessory pathway (Bundle of Kent) between atria and ventricles

- Characteristics: short PR interval; steady rhythm and normal rate (usually); slurred upstroke of the R wave (delta wave); widened QRS complex
- The cardiac impulse can travel in retrograde fashion to the atria over the accessory pathway and initiate a reentrant tachycardia.

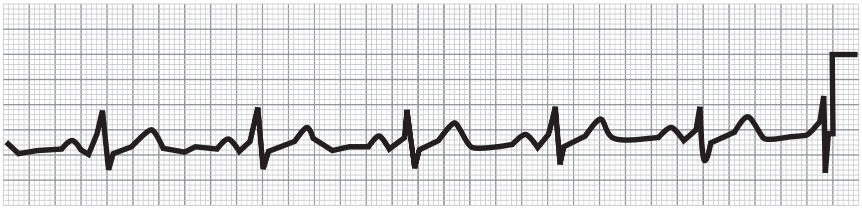


Figure II-3-16. Wolff-Parkinson-White Syndrome

Other Factors Changing the ECG

ST segment changes

- Elevated: transmural infarct or Prinzmetal angina (coronary vasospasm)
- Depressed: subendocardial ischemia or exertional (stable) angina

Potassium

- Hyperkalemia: increases rate of repolarization, resulting in sharp-spiked T waves and a shortened QT interval
- Hypokalemia: decreases rate of repolarization, resulting in U waves and a prolonged QT interval

Calcium

- Hypercalcemia: decreases the QT interval
- Hypocalcemia: increases the QT interval

PART III

Muscle

Excitation-Contraction Coupling 1

Learning Objectives

- ❑ Interpret scenarios on skeletal muscle structure-function relationships
- ❑ Interpret scenarios on regulation of cytosolic calcium
- ❑ Interpret scenarios on altering force in skeletal muscle
- ❑ Interpret scenarios on comparison of striated muscles (skeletal vs. cardiac)
- ❑ Interpret scenarios on smooth muscle function

SKELETAL MUSCLE STRUCTURE–FUNCTION RELATIONSHIPS

Ultrastructure of a Myofibril

A muscle is made up of individual cells called muscle fibers. Longitudinally within the muscle fibers, there are bundles of myofibrils.

- A myofibril can be subdivided into individual sarcomeres. A sarcomere is demarked by Z lines.
- Sarcomeres are composed of filaments creating bands.
- Contraction causes no change in the length of the A band, a shortening of the I band, and a shortening in the H zone (band).
- Titin anchors myosin and is an important component of striated muscle's elasticity.

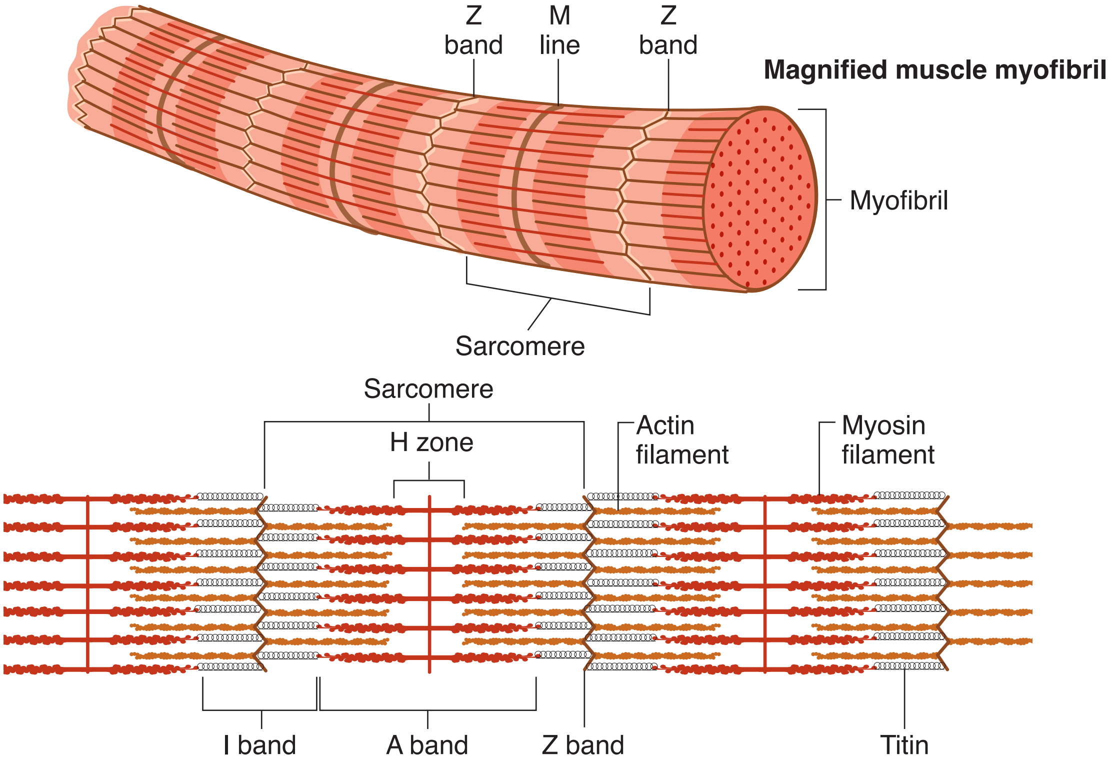


Figure III-1-1. Organization of Sarcomeres

Ultrastructure of the Sarcoplasmic Reticulum

The external and internal membrane system of a skeletal muscle cell is displayed below.

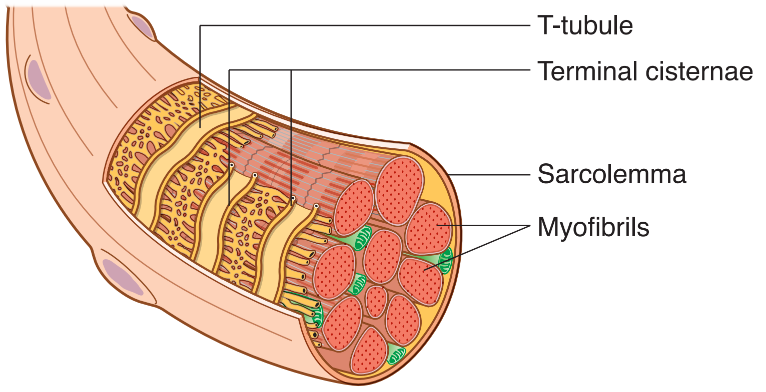


Figure III-1-2. Skeletal Muscle Cell Membranes

T-tubule membranes are extensions of the surface membrane; therefore, the interiors of the T tubules are part of the extracellular compartment.

Terminal cisternae: The sarcoplasmic reticulum is part of the internal membrane system, one function of which is to store calcium. In skeletal muscle, most of the calcium is stored in the terminal cisternae close to the T-tubule system.

Functional Proteins of the Sarcomere

The figure below shows the relationships among the proteins that make up the thin and thick filaments in striated muscle (skeletal and cardiac) and the changes that occur with contraction.

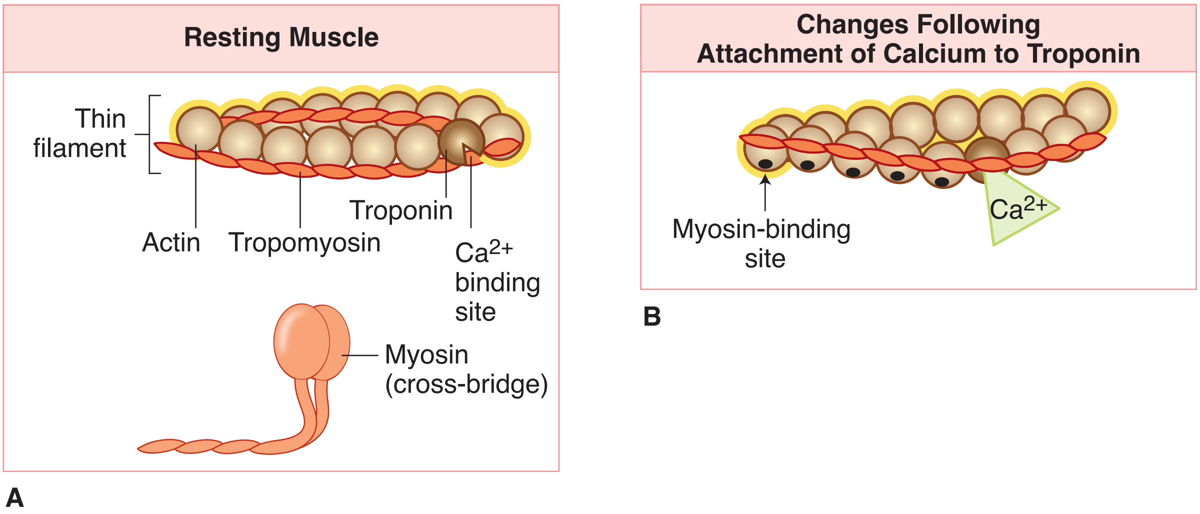


Figure III-1-3. Regulation of Actin by Troponin

Proteins of the thin filaments

- **Actin** is the structural protein of the thin filament. It possesses attachment sites for myosin.
- **Tropomyosin** blocks myosin binding sites on actin.
- **Troponin** is composed of 3 subunits: **troponin-T** (binds to tropomyosin), **troponin-I** (binds to actin and inhibits contraction), and **troponin-C** (binds to calcium).
  - Under resting conditions, no calcium is bound to the troponin, preventing actin and myosin from interacting.
  - When calcium binds to troponin-C, the troponin-tropomyosin complex moves, exposing actin's binding site for myosin. (part B of the figure above)

Proteins of the thick filaments

Myosin has ATPase activity. The splitting of ATP puts myosin in a "high energy" state; it also increases myosin's affinity for actin.

- Once myosin binds to actin, the chemical energy is transferred to mechanical energy, causing myosin to pull the actin filament. This generates active tension in the muscle and is commonly referred to as "the power stroke."
- If the force generated by the power stroke is sufficient to move the load (see next chapter), then the muscle shortens (isotonic contraction).
- If the force generated is not sufficient to move the load (see next chapter), then the muscle doesn't shorten (isometric contraction).

Cross-Bridge Interactions (Chemical-Mechanical Transduction)

Cross-bridge cycling starts when free calcium is available and attaches to troponin, which in turn moves tropomyosin so that myosin binds to actin. Contraction (of a muscle) is the continuous cycling of cross-bridges.

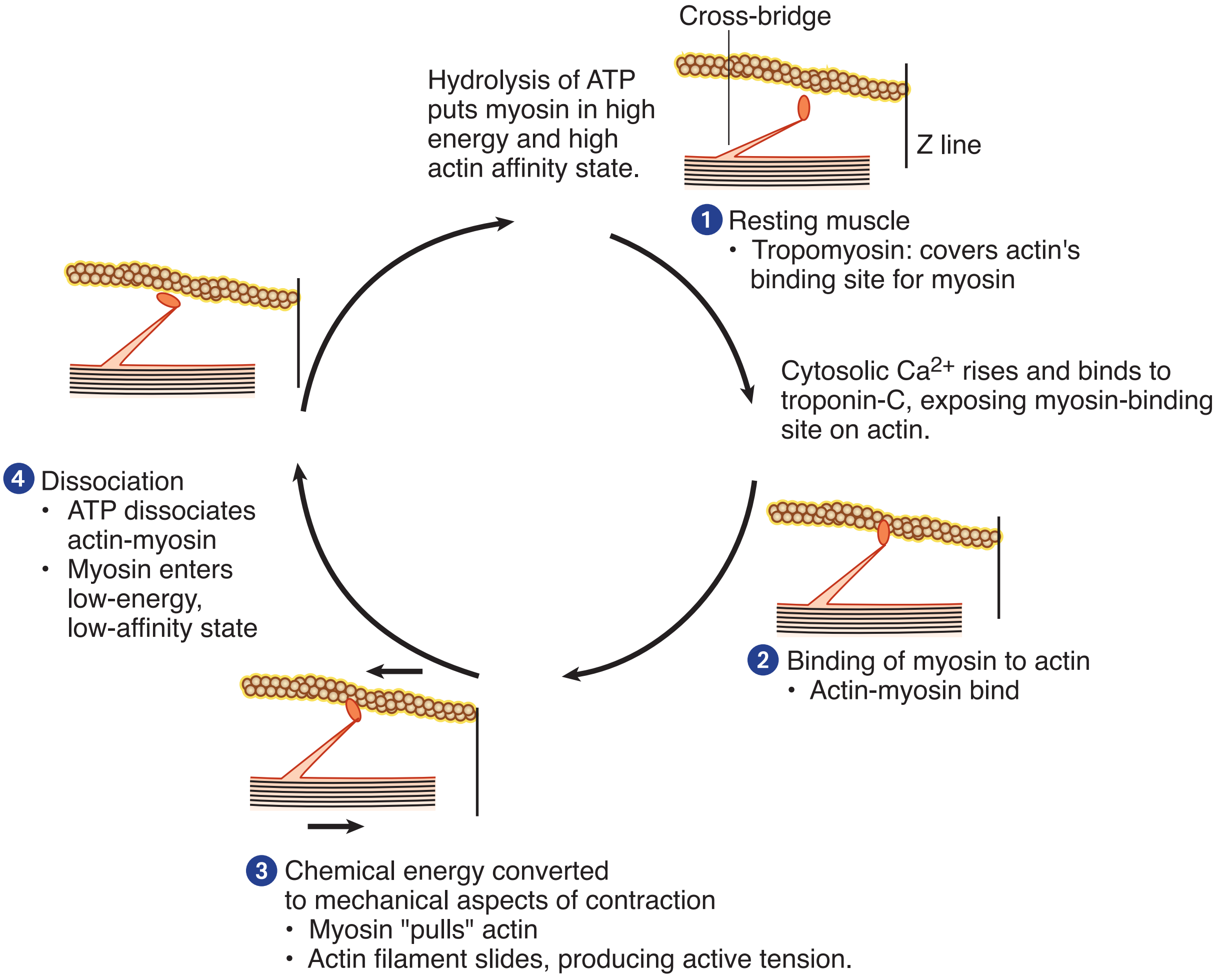


Figure III-1-4. Cross-bridge Cycling During Contraction

ATP is not required to form the cross-bridge linking to actin but is required to break the link with actin. Cross-bridge cycling (contraction) continues until either of the following occurs:

- Withdrawal of Ca^{2+}: cycling stops at position 1 (normal resting muscle)
- ATP is depleted: cycling stops at position 3 (rigor mortis; this would not occur under physiologic conditions)

REGULATION OF CYTOSOLIC CALCIUM

The sarcoplasmic reticulum (SR) has a high concentration of Ca^{2+}. Thus, there is a strong electrochemical gradient for Ca^{2+} to diffuse from the SR into the cytosol.

There are 2 key receptors involved in the flux of Ca^{2+} from the SR into the cytosol: dihydropyridine (DHP) and ryanodine (RyR).

- **DHP** is a voltage-gated Ca^{2+} channel located in the sarcolemmal membrane. Although it is a voltage-gated Ca^{2+} channel, Ca^{2+} **does not** flux through this receptor in skeletal muscle. Rather, DHP functions as a voltage-sensor. When skeletal muscle is at rest, DHP blocks RyR.
- **RyR** is a calcium channel on the SR membrane. When the muscle is in the resting state, RyR is blocked by DHP. Thus, Ca^{2+} is prevented from diffusing into the cytosol.

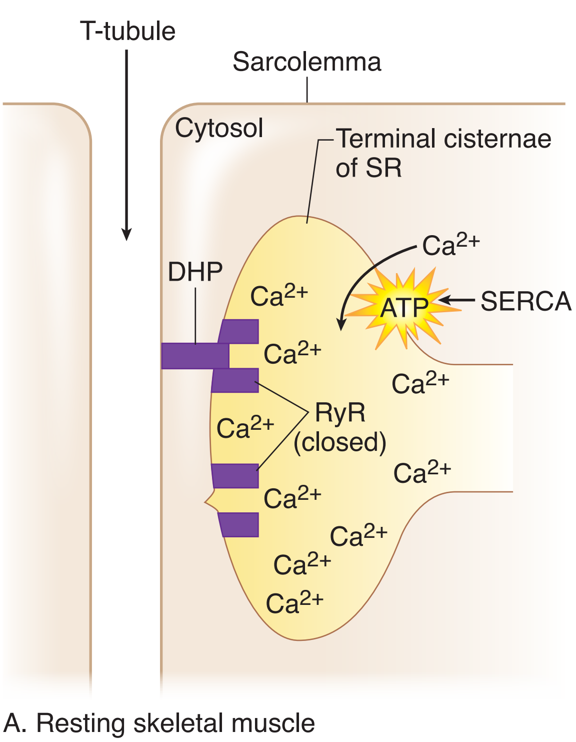


Figure III-1-5. Regulation of Ca^{2+} Release by Sarcoplasmic Reticulum

Sequence

1. Skeletal muscle action potential is initiated at the neuromuscular junction (*see* section II).
2. The action potential travels down the T-tubule.
3. The voltage change causes a conformation shift in DHP (voltage sensor), removing its block of RyR (part B of the figure above).
4. Removal of the DHP block allows Ca^{2+} to diffuse into the cytosol (follows its concentration gradient).
5. The rise in cytosolic Ca^{2+} opens more RyR channels (calcium-induced calcium release).
6. Ca^{2+} binds to troponin-C, which in turn initiates cross-bridge cycle, creating active tension.
7. Ca^{2+} is pumped back into the SR by a calcium ATPase on the SR membrane called sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA).
8. The fall in cytosolic Ca^{2+} causes tropomyosin to once again cover actin's binding site for myosin and the muscle relaxes, provided of course ATP is available to dissociate actin and myosin.

Key Points

- Contraction-relaxation states are determined by cytosolic levels of Ca^{2+}.
- The source of the calcium that binds to the troponin-C in skeletal muscle is solely from the cell's sarcoplasmic reticulum. Thus, no extracellular Ca^{2+} is involved.
- Two ATPases are involved in contraction:
  - **Myosin ATPase** supplies the energy for the mechanical aspects of contraction by putting myosin in a high energy and affinity state.
  - **SERCA** pumps Ca^{2+} back into the SR to terminate the contraction, i.e., causes relaxation.

ALTERING FORCE IN SKELETAL MUSCLE

Mechanical Response to a Single Action Potential

The figure below illustrates the mechanical contraction of skeletal muscle and the action potential on the same time scale. Note the sequence of events: action potential causes Ca^{2+} release. The release of Ca^{2+} evokes a muscle contraction (twitch).

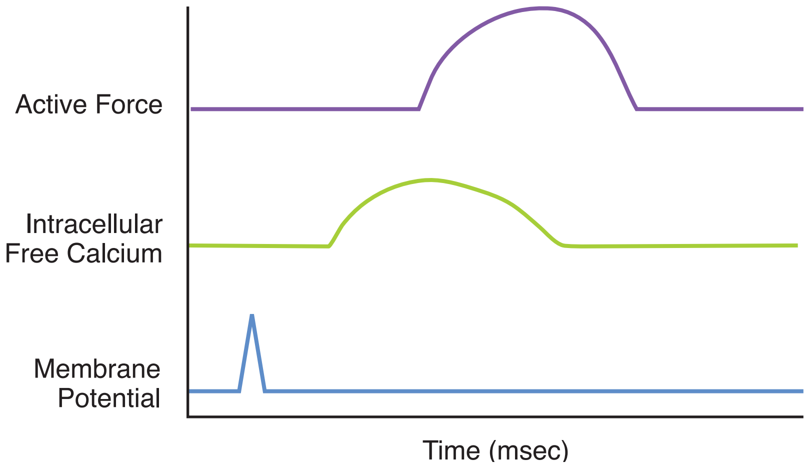


Figure III-1-6. Time Course of Events During Contraction

The muscle membrane has completely repolarized well before the start of force development.

Summation and Recruitment

Under normal circumstances, enough Ca^{2+} is released by a single muscle action potential to completely saturate all the troponin-C binding sites. This means that all available cross-bridges are activated and thus force cannot be enhanced by increasing cytosolic Ca^{2+}.

Instead, peak force in skeletal muscle is increased in 2 ways: summation and recruitment.

Summation

- Because the membrane has repolarized well before force development, multiple action potentials can be generated prior to force development.
- Each action potential causes a pulse of Ca^{2+} release.
- Each pulse of Ca^{2+} initiates cross-bridge cycling and because the muscle has not relaxed, the mechanical force adds onto (summates) the force from the previous action potential (Figure III-1-7).
- This summation can continue until the muscle tetanizes in which case there is sufficient free Ca^{2+} so that cross-bridge cycling is continuous.

Recruitment

- A single alpha motor neuron innervates multiple muscle fibers. The alpha motor neuron and all the fibers it innervates is called a motor-unit.
- Recruitment means activating more motor units, which in turn engage more muscle fibers, causing greater force production.

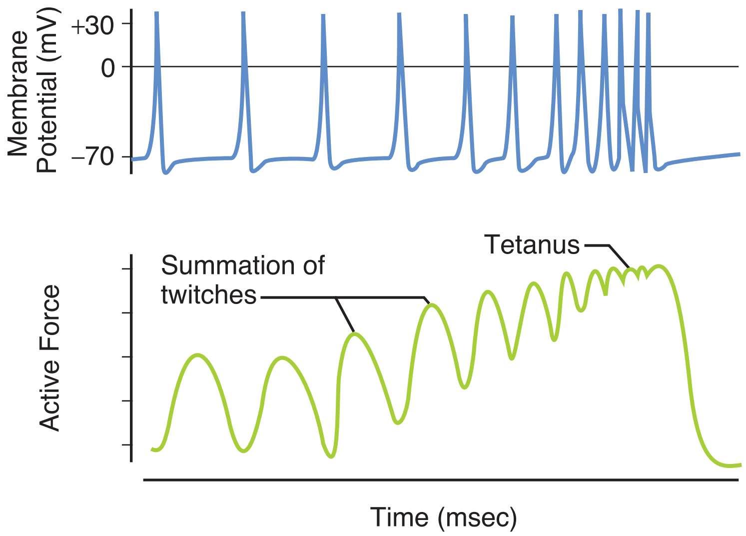


Figure III-1-7. Summation of Individual Twitches and Fusion into Tetanus

Recall Question

Which of the following is the mechanism of action of rigor mortis?

A. Withdrawal of $Ca2^{+}$ which stops cycling at position 1

B. Cytosolic calcium rises and binds to troponin-C, exposing myosin-binding site on actin

C. Depletion of ATP which stops cycling at position 3

D. Depletion of calcium which stops cycling at position 3

E. Depletion of actin-myosin cross bridging which stops cycling at position 3

Answer: C

COMPARISON OF STRIATED MUSCLES

Skeletal and **cardiac muscle** are both striated muscle and share many similarities. Nevertheless, there are important differences.

Similarities

- Both have the same functional proteins, i.e., actin, tropomyosin, troponin, myosin, and titin.
- A rise in cytosolic Ca^{2+} initiates cross-bridge cycling thereby producing active tension.
- ATP plays the same role.
- Both have SERCA.
- Both have RyR receptors on the SR and thus show calcium-induced calcium release.

Differences

- Extracellular Ca^{2+} is involved in cardiac contractions, but not skeletal muscle. This extracellular Ca^{2+} causes calcium-induced calcium release in cardiac cells.
- Magnitude of SR Ca^{2+} release can be altered in cardiac (see section on cardiac mechanics), but not skeletal muscle.
- Cardiac cells are electrically coupled by gap junctions, which do not exist in skeletal muscle.
- Cardiac myocytes remove cytosolic Ca^{2+} by 2 mechanisms: SERCA and a Na^+—Ca^{2+} exchanger (3 Na^+ in, 1 Ca^{2+} out) on the sarcolemmal membrane. Skeletal muscle only utilizes SERCA.

Bridge to Pathology

Dysfunction in the titin protein has been associated with dilated and restrictive cardiomyopathies (see next section).

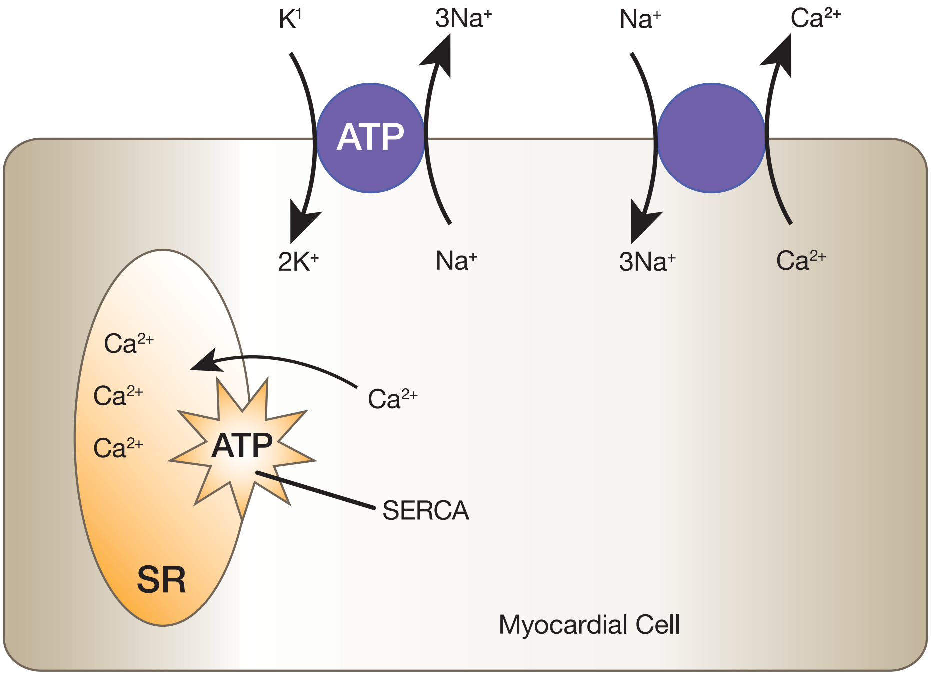


Figure III-1-8. Removal of Cytosolic Calcium in Myocardial Cells

- Cardiac cells have a prolonged action potential. The figure above illustrates that the twitch tension is already falling (muscle starting to relax) while the action potential is still in the absolute refractory period. Thus, a second action potential cannot be evoked before the mechanical event is almost completed. This approximately equal mechanical and electrical event prevents summation of the force and if the muscle can't summate, it can't tetanize.

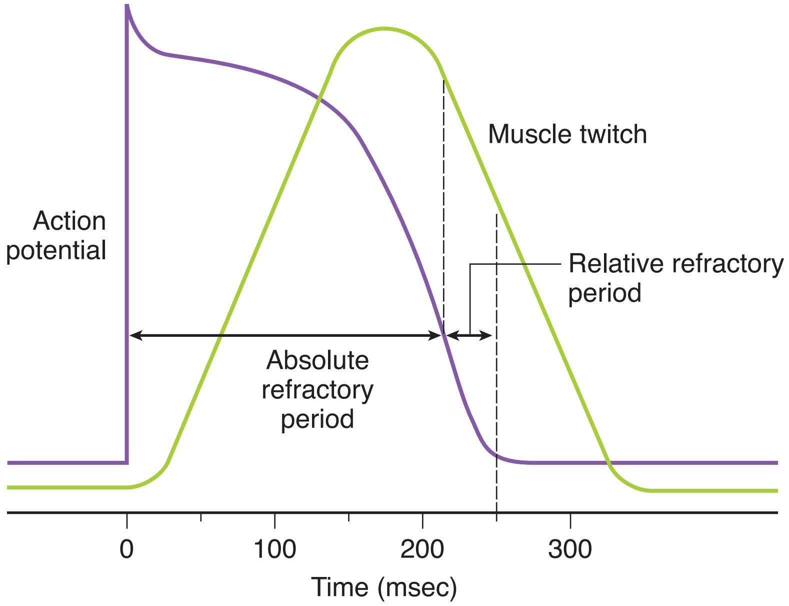
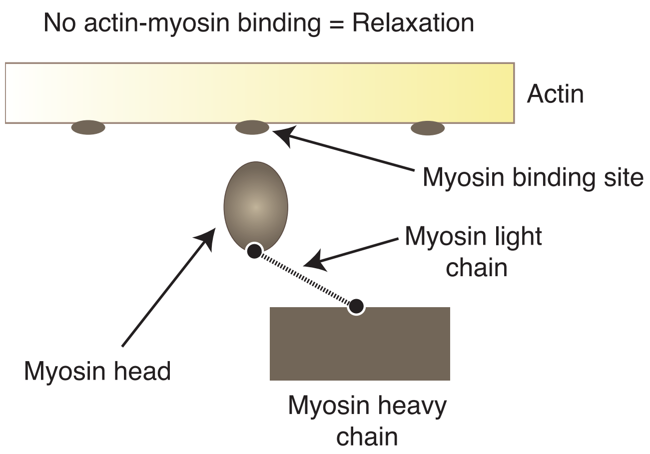


Figure III-1-9. Force and Refractory Periods

SMOOTH MUSCLE

Actin-Myosin Interaction



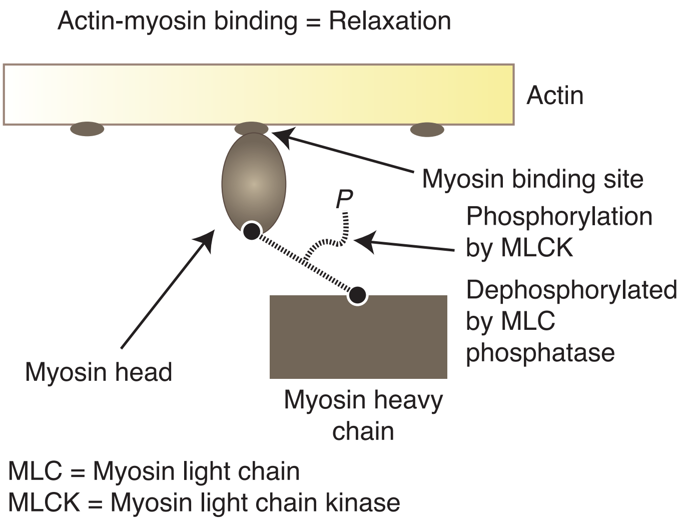


Figure III-1-10a. Relaxed Smooth Muscle

Figure III-1-10b. Contracted Smooth Muscle

- In contrast to striated muscle, smooth muscle lacks tropomyosin, troponin, and titin.
- Similar to striated muscle, the binding of actin and myosin produces tension.
- In the resting state, MLC is not phosphorylated and has very low affinity for actin. Thus they do not interact and smooth muscle is relaxed (Figure III-1-10a)
- On the other hand, phosphorylation of MLC puts myosin in a high-affinity state for actin, resulting in the binding of actin and myosin to produce a power stroke (Figure III-1-10b).
- MLC is phosphorylated by myosin light-chain kinase (MLCK) and dephosphorylated by MLC phosphatase.
- Similar to striated muscle, the trigger for contraction is increasing cytosolic calcium, which activates MLCK

Regulation of Smooth Muscle

- Voltage-gated calcium channels (L-type) reside in the sarcolemma of smooth muscle. Depolarization opens these channels, resulting in calcium influx into the cytosol. This calcium triggers calcium release from the SR (calcium-induced calcium release, similar to cardiac muscle).
- Increasing IP3 also evokes calcium efflux from the SR. IP3 is increased by an agonist binding a Gq coupled receptor (e.g., the alpha-1 receptor).
- This cytosolic calcium binds to the protein calmodulin (CAM). This calcium-calmodulin complex activates MLCK, which in turn phosphorylates MLC.
- As indicated above, phosphorylation of MLC causes binding of actin and myosin, in turn eliciting a contraction of smooth muscle.
- Although not illustrated in Figure III-1-11, similar to striated muscle (see above), ATP dissociates actin and myosin. If MLC remains phosphorylated, then actin and myosin rebind to produce tension (similar to cross-bridge cycling described above for striated muscle).
- MLC phosphatase dephosphorylates myosin, reducing the affinity of myosin for actin, causing relaxation.
- When cytosolic calcium is high, MLCK dominates. When cytosolic calcium is low, MLC phosphatase dominates.
- Smooth muscle reduces cytosolic calcium via the same mechanisms described above for cardiac cells.

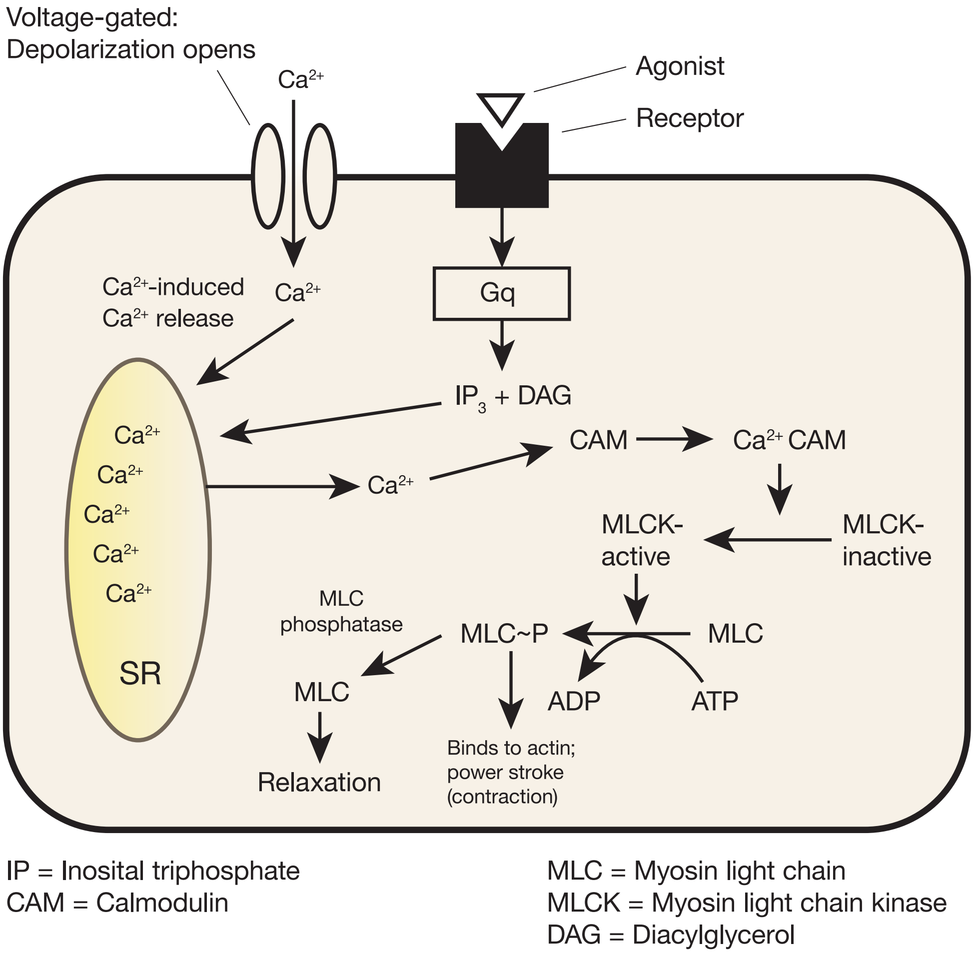


Figure III-1-11. Smooth Muscle Cell

Skeletal Muscle Mechanics 2

Learning Objectives

- ❑ Use knowledge of overview of muscle mechanics
- ❑ Interpret scenarios on length-tension curves
- ❑ Use knowledge of relationship between velocity and load
- ❑ Demonstrate understanding of properties of white vs. red muscle
- ❑ Solve problems concerning comparison of muscle types

MUSCLE MECHANICS

Preload

Preload is the load on a muscle in a relaxed state, i.e., before it contracts. Applying preload to muscle does 2 things:

- Stretches the muscle: This in turn, stretches the sarcomere. **The greater the preload, the greater the stretch of the sarcomere.**
- Generates passive tension in the muscle: Muscle is elastic (see titin, previous chapter) and thus "resists" the stretch applied to it. Think of the "snap-back" that occurs when one stretches a rubber band. The force of this resistance is measured as passive tension. **The greater the preload, the greater the passive tension in the muscle.**

Afterload

Afterload is the load the muscle works against. If one wants to lift a 10 kg weight, then this weight represents the afterload. Using the 10 kg weight example, 2 possibilities exist:

- If the muscle generates more than 10 kg of force, then the weight moves as the muscle shortens. This is an **isotonic contraction**.
- If the muscle is unable to generate more than 10 kg of force, then the muscle won't shorten. This is an **isometric contraction**.
- Types of tension
  - Passive: produced by the preload
  - Active: produced by cross-bridge cycling
  - Total: sum of active and passive tension

LENGTH–TENSION CURVES

Length–tension curves are important for understanding both skeletal and cardiac muscle function. The graphs that follow are all generated from skeletal muscle in vitro, but the information can be applied to both skeletal muscle and heart muscle in vivo.

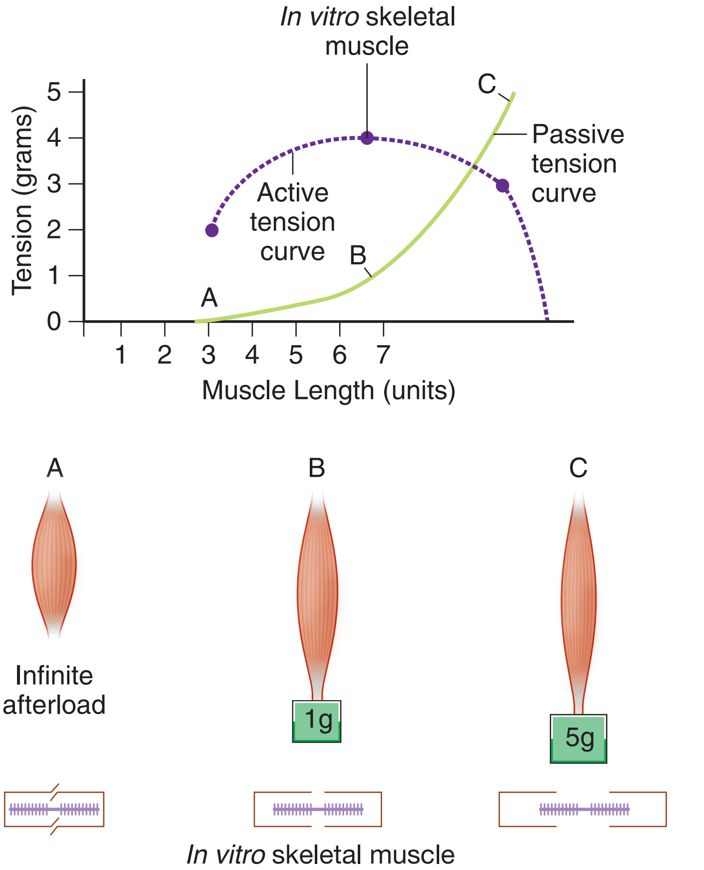
Passive Tension Curve

As seen in the figure below, the green line shows that muscle behaves like a rubber band. The elastic properties of the muscle resist this stretch and the resulting tension is recorded. There is a direct (non-linear) relationship between the degree of stretch and the passive tension created that resists this stretch.

Point A: no preload, thus no stretch and no passive tension

Point B: preload of 1 g stretches muscle, thus increasing its resting length, resulting in ~1 g of passive tension

Point C: preload of 5 g increases muscle stretch, producing a greater resting length and thus a greater passive tension



Passive tension = 0
Active tension = 2
Total tension = 2

Passive tension = 1
Active tension = 4
Total tension = 5

Passive tension = 5
Active tension = 3
Total tension = 8

Figure III-2-1. Preload, Active and Passive Tension: The Length–Tension Relationship

Active Tension

In the figure above, the purple line shows the tension developed by stimulating the muscle to contract at the different preloads. In this example, the contraction is a **maximal isometric contraction**, i.e., the contraction produces tension, but the afterload is much greater than the tension the muscle develops and thus the muscle doesn't shorten. Recall that active tension represents the force generated by cross-bridge cycling. It is important to note the shape (bell-shaped) of the active tension curve.

- **Preload of A**: When there is no preload, the evoked muscle contraction develops ~2 g of active tension.
- **Preload of B**: At this preload, the active tension produced by stimulation of the muscle is greater, ~4 g.
- **Preload of C**: This preload results in less active tension than the previous preload. Thus, active tension increases as the muscle is stretched, up to a point. If stretched beyond this point, then active tension begins to fall.
- **Optimal length (L_o)**: L_o represents the muscle length (preload) that produces the greatest active tension. (In the figure above, this occurs at the preload designated by B.)

Explanation of Bell-shaped Active Tension Curve

The same figure above shows a simplified picture of a sarcomere. Actin is the thin brown line, while myosin is depicted in purple. The magnitude of active tension depends on the number of actin-myosin cross-bridges that can form (directly related).

- Preload A: actin filaments overlap
  - Thus, the force that can be exerted by myosin tugging the actin is compromised and the active tension is less.
- Preload B (L_o): all myosin heads can bind to actin, and there is separation of actin filaments
  - Thus, active tension generated is greatest here because there is optimal overlap of actin and myosin.
- Preload C: the stretch is so great that actin has been pulled away from some of the myosin filament, and thus fewer actin-myosin interactions are available, resulting in diminished active tension.
  - If taken to the extreme, greater stretch could pull actin such that no actin-myosin interactions can occur, and thus no active tension results (active tension curve intersects the x-axis). This is an experimental, rather than physiologic phenomenon.
- Total tension: sum of passive and active tension (bottom of figure above)

RELATIONSHIP BETWEEN VELOCITY AND LOAD

As seen in the figure below, the maximum velocity of shortening (Vmax) occurs when there is no afterload on the muscle. Increasing afterload decreases velocity, and when afterload exceeds the maximum force generated by the muscle, shortening does not occur (isometric contraction).

*Maximum velocity (Vmax) is determined by the muscle's ATPase activity. It is the ATPase activity that determines a fast versus a slow muscle.

**Maximum force generated by a muscle occurs when summation is maximal (complete summation) and all motor units for the given muscle are fully recruited. The absolute amount of force is directly related to muscle mass and preload, with the greatest force occurring when the preload is at L_0.

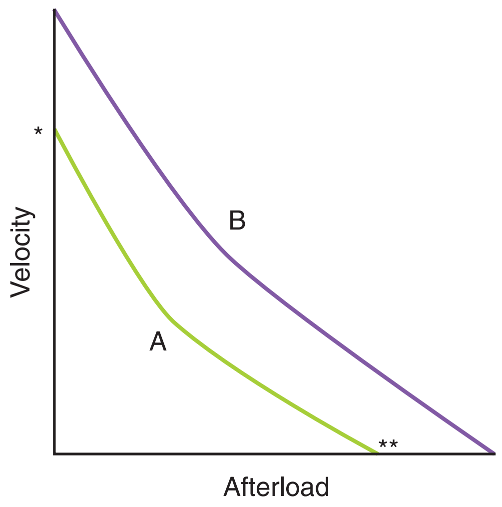


Figure III-2-2. Force–Velocity Curve

In the figure above, **muscle A** is a smaller, slower muscle (red muscle), while **muscle B** is a larger, faster muscle (white muscle).

As load increases, the distance shortened during a single contraction decreases. So, with increased afterload, both the velocity of contraction and the distance decrease.

PROPERTIES OF WHITE VS. RED MUSCLE

White Muscle

Generally, white muscle is the large (powerful) muscle that is utilized short-term, e.g., ocular muscles, leg muscles of a sprinter. Major characteristics are as follows:

- Large mass per motor unit
- High ATPase activity (fast muscle)
- High capacity for anaerobic glycolysis
- Low myoglobin

Red Muscle

Generally, red muscle is the smaller (less powerful) muscle utilized long-term (endurance muscle), e.g., postural muscle. Major characteristics are as follows:

- Small mass per motor unit
- Lower ATPase activity (slower muscle)
- High capacity for aerobic metabolism (mitochondria)
- High myoglobin (imparts red color)

Recall Question

Which of the following is a characteristic of white muscle?

A. It is reponsible for slower muscle movements.

B. It has a high mitochondria content.

C. It primarily utilizes aerobic metabolism.

D. It has a greater mass per motor unit.

E. It contains high amounts of myoglobin.

Answer: D

PART IV

Cardiovascular

Hemodynamics and Important Principles 1

Learning Objectives

- ❑ Answer questions about systolic performance of the ventricle
- ❑ Explain information related to ventricular function curves
- ❑ Solve problems concerning chronic changes: systolic and diastolic dysfunction

THE CARDIOVASCULAR SYSTEM

Cardiac Output

The cardiovascular system consists of 2 pumps (left and right ventricles) and 2 circuits (pulmonary and systemic) connected in series.

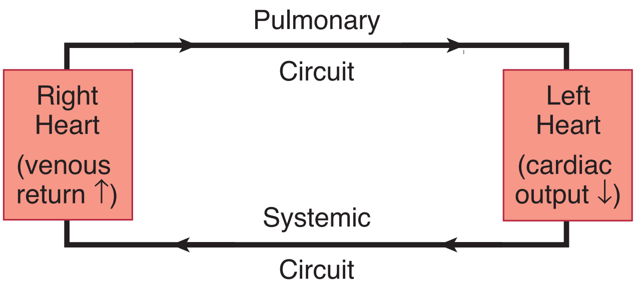


Figure IV-1-1. The Circulatory System

When circuits are connected in series, flow must be equal in the 2 circuits.

- Cardiac output is the output of either the left or right ventricle, and because of the series system, they are equal.
- The chemical composition of pulmonary venous blood (high oxygen, low carbon dioxide) is very close to the chemical composition of systemic arterial blood.
- Systemic mixed venous blood entering the right atrium has the same composition (low oxygen, high carbon dioxide) as pulmonary arterial blood.

Note

The function of the heart is to transport blood and deliver oxygen in order to maintain adequate tissue perfusion. It also removes waste products, e.g., CO_2 created by tissue metabolism.

Because the heart is a "demand pump" that pumps out whatever blood comes back into it from the venous system, it is effectively the amount of blood returning to the heart which determines how much blood the heart pumps out.

Structure–Function Relationships of the Systemic Circuit

The systemic circuit is a branching circuit. It begins as a large single vessel, the aorta, and branches extensively into progressively smaller vessels until the capillaries are reached. The reverse then takes place in the venous circuit.

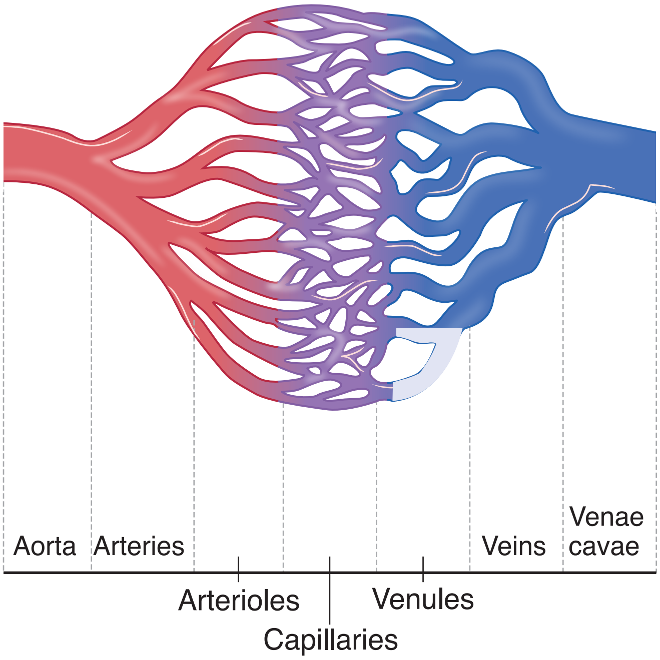


Figure IV-1-2. Organization of Systemic Vessels

HEMODYNAMICS

Pressure, Flow, Resistance

The Poiseuille equation represents the relationship of flow, pressure, and resistance.

$$Q = \frac{P_1 - P_2}{R}$$

It can be applied to a single vessel, an organ, or an entire circuit.

Q: flow (mL/min)

P_1: upstream pressure (pressure head) for segment or circuit (mm Hg)

P_2: pressure at the end of the segment or circuit (mm Hg)

R: resistance of vessels between P_1 and P_2 (mm Hg/mL/min)

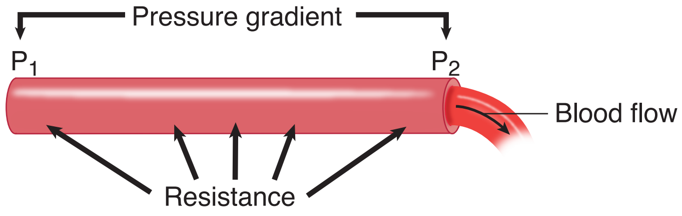


Figure IV-1-3. Poiseuille Equation Applied to Single Vessel

The flow to an organ such as the kidney, for example, could be calculated as mean arterial pressure minus renal venous pressure divided by the resistance of all vessels in the renal circuit.

Determinants of resistance

$$\text{Resistance} = \frac{P_1 - P_2}{Q}$$

$$\text{Units of Resistance} = \frac{\text{mm Hg}}{\text{mL/min}} = \frac{\text{pressure}}{\text{volume/time}}$$

The resistance of a vessel is determined by 3 major variables: $R \propto \frac{\nu L}{r^4}$

Vessel radius (r) is the most important factor determining resistance. If resistance changes, then the following occurs:

- Increased resistance decreases blood flow, increases upstream pressure, and decreases downstream pressure.
- Decreased resistance increases blood flow, decreases upstream pressure and increases downstream pressure.
- The pressure "drop" (difference between upstream and downstream) is directly related to the resistance. There is a big pressure drop when resistance is a high and minimal pressure drop when resistance is a low.

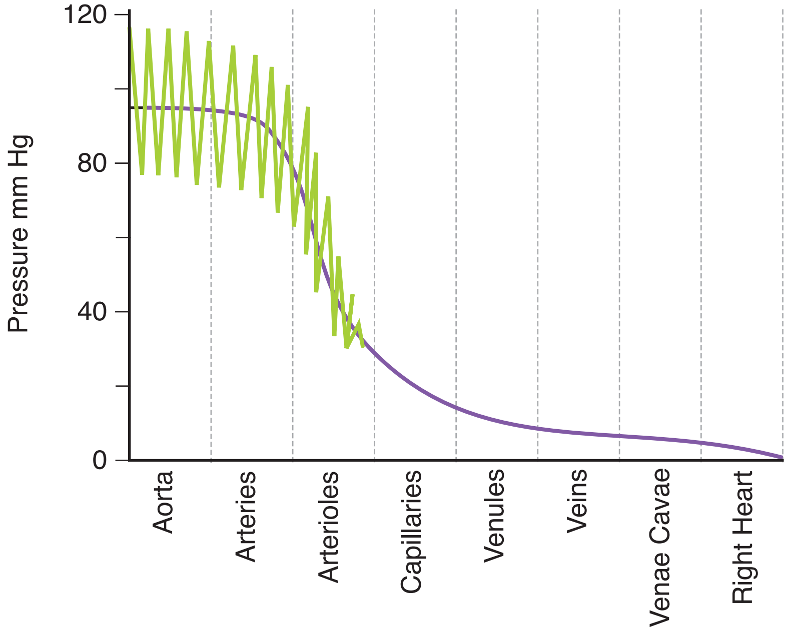


Figure IV-1-4. Systemic System Pressures

Whole body application of resistance

The figure above shows, in a horizontal subject, the phasic and mean pressures from the aorta to the vena cava.

- Mean arterial pressure (MAP) is measured in the aorta and is about 93 mm Hg (time weighted average because more time is spent in diastole). This represents the pressure head (upstream pressure) for the systemic circulation.
- The pressure dissipates as the blood flows down the circulatory tree because of resistance. The amount of pressure lost in a particular segment is proportional to the resistance of that segment.
- There is a small pressure drop in the major arteries (low-resistance segment); the largest drop is across the arterioles (highest resistance segment), and another small pressure drop occurs in the major veins (low-resistance segment).
- Since the largest pressure drop across the systemic circulation occurs in arterioles, they are the main site resistance. This resistance is called total peripheral resistance (TPR) or systemic vascular resistance (SVR).
- TPR/SVR is afterload to the heart (see next chapter).

Blood viscosity (*v*) is a property of a fluid that is a measure of the fluid's internal resistance to flow. The **greater the viscosity**, the **greater the resistance**.

The prime determinant of blood viscosity is the hematocrit.

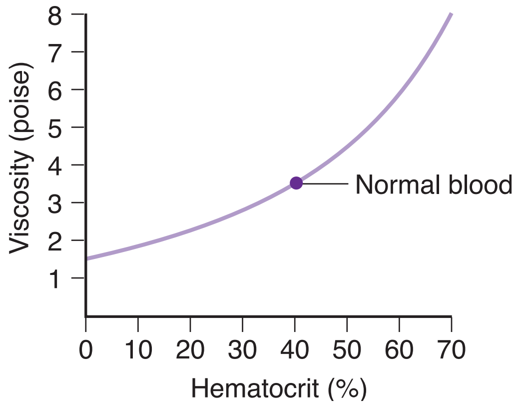


Figure IV-1-5. Effect of Hematocrit on Blood Viscosity

Anemia decreases viscosity. Polycythemia increases viscosity.

Vessel length (L)

The **greater the length**, the **greater the resistance**.

- If the length doubles, the resistance doubles.
- If the length decreases by half, the resistance decreases by half.
- Vessel length is constant; therefore, changes in length are not a physiologic factor in regulation of resistance, pressure, or flow.

Note

If a blood sample from an adult is centrifuged in a graduated test tube, the relative volume of packed red cells is called the **hematocrit**. For a normal adult this volume is about 40–45% of the total, meaning the red cells occupy about 40–45% of the blood in the body.

The white blood cells are less dense than the red blood cells and form a thin layer (the so-called *buffy coat*). That is why hematocrit is a major determinant of blood viscosity.

Velocity

Velocity is the rate at which blood travels through a blood vessel. Mean linear velocity is equal to flow divided by the cross-sectional area (CSA). Thus, velocity is directly related to flow, but if CSA changes then velocity is affected. The important functional applications of this are:

- CSA is high in capillaries, but low in the aorta.
- Velocity is therefore high in the aorta and low in the capillaries.
- The functional consequence of this is that low velocity in the capillaries optimizes exchange.
- The potential pathology of this is that because the aorta has high velocity and a large diameter, turbulent blood flow can occur.

Note

Although **velocity is directly related to blood flow,** it is different in that it refers to a **rate,** e.g., cm/sec.

Laminar versus Turbulent Flow

There can be 2 types of flow in a system: laminar and turbulent.

Laminar flow is flow in layers. It occurs throughout the normal cardiovascular system, excluding flow in the heart. The layer with the highest velocity is in the center of the tube.

Turbulent flow is nonlayered flow. It creates murmurs. These are heard as bruits in vessels with severe stenosis.

Turbulent flow produces more resistance than laminar flow.

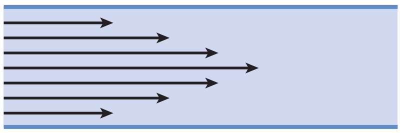


Figure IV-1-6. Laminar Flow

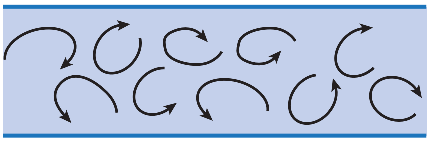


Figure IV-1-7. Turbulent Flow

Relation of Reynold's number to laminar and turbulent flow

$$\text{Reynold's number} = \frac{(\text{diameter})(\text{velocity})(\text{density})}{\text{viscosity}}$$

$>2{,}000$ = turbulent flow

$<2{,}000$ = laminar flow

The number inducing turbulence is not absolute. For example, atherosclerosis reduces the Reynold's number at which turbulence begins to develop in the systemic arteries. In addition, thrombi are more likely to develop with turbulent flow than in a laminar flow system.

The following promote the development of turbulent flow (i.e., increase Reynolds' number):

- Increasing tube diameter
- Increasing velocity
- Decreasing blood viscosity, e.g., anemia (cardiac flow murmur)

The vessel in the systemic circuit that is closest to the development of turbulent flow is the aorta. It is a large-diameter vessel with high velocity. This is where turbulence should appear first in anemia.

The following also promote turbulence:

- Vessel branching
- Narrow orifice (severe stenosis)—due to very high velocity of flow

During inspiration and expiration, turbulent flow occurs in the large airways of the conducting zone.

Series Versus Parallel Circuits

- If resistors are in series, then the total resistance is the sum of each individual resistor. **RT = R1 + R2 + R3…**
- If resistors are in parallel, then the total resistance is added as reciprocals of each resistor. **1/RT = 1/R1 + 1/R2 + 1/R3…**
- Thus, total resistance is less in parallel circuits compared to series circuits.

The application of this concept is that blood flow to the various organ beds of the systemic circulation is the result of parallel branches off of the aorta. Because they are parallel branches, the total resistance of the systemic circulation is **less than** if the organs were in series bloodflow-wise (note figure below).

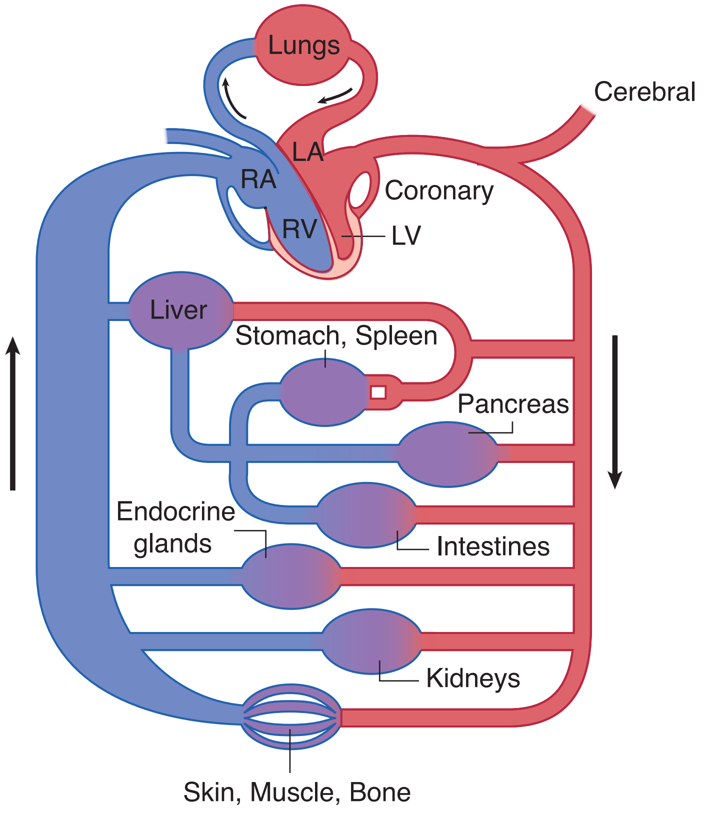


Figure IV-1-8. Systemic Circuit

VESSEL COMPLIANCE

$$C = \frac{\Delta V}{\Delta P}$$

Compliance of a vessel can be calculated, but the resulting number is, for all practical purposes, meaningless. It is much more important to simply have a good concept of compliance and understand the differences in compliance among the vessels that make up the cardiovascular system.

- Compliance is essentially how easily a vessel is stretched. If a vessel is easily stretched, it is considered very compliant. The opposite is noncompliant or stiff.
- Elasticity is the inverse of compliance. A vessel that has high elasticity (a large tendency to rebound from a stretch) has low compliance.

Systemic Veins

Systemic veins are about 20 times more compliant than systemic arteries.

- Veins also contain about 70% of the systemic blood volume and thus represent the major blood reservoir.
- If blood is in the veins, then it is not available for the heart to pump and is thus not contributing to the circulating blood volume.

In short: When considering whole-body hemodynamics, compliance resides in the venous system. One must not forget the functional implications of arterial compliance, particularly with respect to arterial pressures (see below), but for the circulation as a whole, compliance is in the venous system.

WALL TENSION

LaPlace relationship:

$$\mathbf{T \propto Pr}$$

The aorta is the artery with the greatest wall tension (greatest pressure and radius).

T: wall tension

P: pressure

r: radius

Development of an Arterial Aneurysm

A developing arterial aneurysm can be seen below. The pressures at points A, B, and C will be approximately the same.

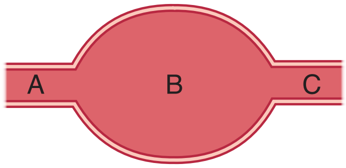


Figure IV-1-9. Aortic Enlargement

- Thus, because the aneurysm has a greater radius, its wall tension is greater than that of the surrounding normal vessel segments.
- Also, as the aneurysm enlarges, wall tension increases and the vessel is more likely to burst. Examples are subarachnoid hemorrhage, aortic aneurysm, and diverticulitis.
- Another type of aneurysm is a dissecting aneurysm. In systemic arterial disease, the high velocity in the aorta may damage the endothelial lining, allowing blood to flow between and dissect the layers of the aorta. This weakens the aortic wall and is considered a life-threatening condition.
- This principle also is important in dilated heart failure, in which the increased chamber size places greater tension on the failing ventricle. This further reduces its performance.

Cardiac Muscle Mechanics 2

Learning Objectives

- ❏ Describe how preload, contractility, and afterload affect systolic performance of the ventricle
- ❏ Predict movement of a point on a ventricular function curve due to physiologic and disease changes
- ❏ Describe examples of chronic pressure and volume overload

SYSTOLIC PERFORMANCE OF THE VENTRICLE

Systolic performance means the overall force generated by the ventricular muscle during systole. The heart does 2 things in systole: pressurizes and ejects blood.

An important factor influencing systolic performance is the number of cross-bridges cycling during contraction. The **greater the number of cross-bridges cycling, the greater the force of contraction**.

Systolic performance is determined by 3 independent variables:

- Preload
- Contractility
- Afterload

These 3 factors are summed together to determine the overall systolic performance of the ventricle. Recent work has demonstrated that they are not completely independent, but the generalizations made here will apply to the physiologic and clinical setting.

Preload

As in skeletal muscle, preload is the load on the muscle in the relaxed state.

More specifically, it is the load or prestretch on ventricular muscle at the end of diastole.

Preload on ventricular muscle is not measured directly; rather, indices are utilized.

The best indices of preload on ventricular muscle are those measured directly in the ventricles. Indices of left ventricular preload:

- Left ventricular end-diastolic volume (LVEDV)
- Left ventricular end-diastolic pressure (LVEDP)

Possibly somewhat less reliable indices of left ventricular preload are those measured in the venous system.

- Central venous pressure (CVP)
- Pulmonary capillary wedge pressure (PCWP)
- Right atrial pressure (RAP)

Pulmonary wedge pressure, sometimes called pulmonary capillary wedge pressure, is measured from the tip of a Swan-Ganz catheter, which, after passing through the right heart, has been wedged in a small pulmonary artery. The tip is pointing downstream toward the pulmonary capillaries, and the pressure measured at the tip is probably very close to pulmonary capillary pressure, which is very close to left atrial pressure. A rise in pulmonary capillary wedge pressure is evidence of an increase in preload on the left ventricle. In some cases, such as in mitral stenosis, it is not a good index of left ventricular preload.

Along similar lines, measurement of systemic central venous pressure is used as an index of preload.

Preload factor in systolic performance (Frank-Starling mechanism)

The preload effect can be explained on the basis of a change in sarcomere length.

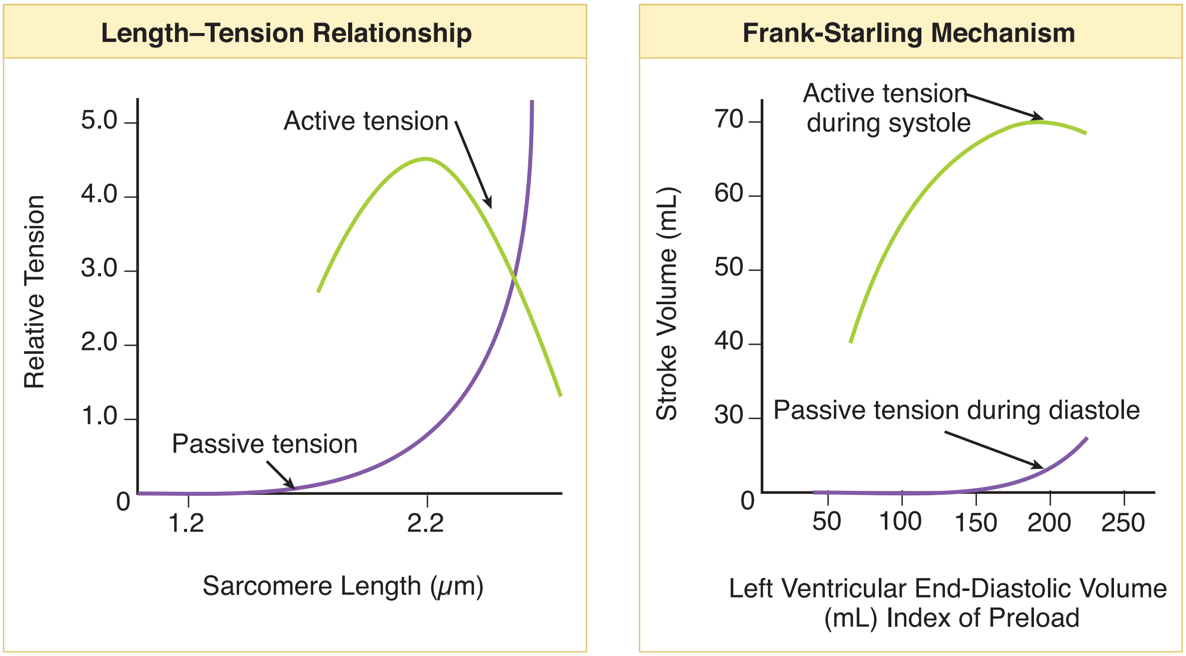


Figure IV-2-1. Length–Tension Relationships in Skeletal and Cardiac Muscle

The resting length of skeletal muscle *in vivo* is at a sarcomere length close to the optimum for maximal cross-bridge linking between actin and myosin during contraction (L_o).

Heart muscle at the end of diastole is below this point. Thus, in a normal heart, increased preload increases sarcomere length toward the optimum actin-myosin overlap. This results in more cross-linking and a more forceful contraction during systole.

Contractility (Inotropic State)

An acceptable definition of **contractility** would be a change in performance at a given preload and afterload. Thus, contractility is a change in the force of contraction at any given sarcomere length.

- Acute changes in contractility are due to changes in the intracellular dynamics of calcium.
- Drugs which increase contractility usually provide more calcium and at a faster rate to the contractile machinery.
- More calcium increases the availability of cross-link sites on the actin, increasing cross-linking and the force of contraction during systole.
- Calcium dynamics do not explain chronic losses in contractility, which in most cases are due to overall myocyte dysfunction.

Indices of contractility

Increased ejection fraction (stroke volume/end-diastolic volume). Ejection fraction can now be estimated fairly easily by a noninvasive technique and is currently a common clinical index of contractility. There is no ideal index of contractility. Ejection fraction is influenced by afterload, but in most cases an increase in contractility is accompanied by an increase in ejection fraction.

Note that ejection fraction simply indicates the percentage of blood ejected from the ventricle; it does not by itself give information about preload or stroke volume.

When contractility increases, there are changes in addition to an increased force of contraction.

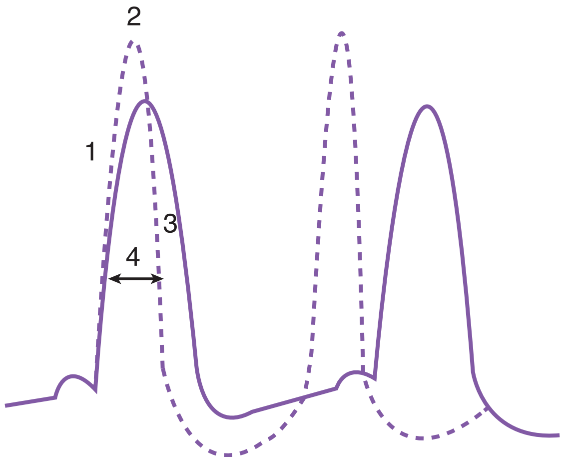


Figure IV-2-2. Effects of Increased Contractility

Solid line = left ventricular pressure before (and the dashed line after) an increase in contractility via increased sympathetic stimulation

The numbers refer to the descriptions after the figure.

The overall changes induced by increased contractility can be summarized as follows:

1. increased *dp/dt* (change in pressure vs change in time): increased slope, thus increased rate of pressure development
2. increased peak left ventricular pressure due to a more forceful contraction
3. increased rate of relaxation due to increased rate of calcium sequestration
4. decreased systolic interval due to effects #1 and #3

Both an increased preload and an increased contractility are accompanied by an increased peak left ventricular pressure, but only with an increase in contractility is there a decrease in the systolic interval.

Whereas contractility affects systolic interval, heart rate determines diastolic interval. Thus, increased sympathetic activity to the heart produces the following:

- Systolic interval decreased: contractility effect
- Diastolic interval decreased: heart rate effect

A high heart rate (pacemaker-induced) produces a small increase in contractility (Bowditch effect). Because Ca^{2+} enters the cell more rapidly than it is sequestered by the sarcoplasmic reticulum, intracellular Ca^{2+} increases. The increased contractility helps compensate for the reduced filling time associated with high heart rates.

Afterload

Afterload is the "load" against which the heart must eject blood. Exactly what constitutes afterload to the heart is the subject of much debate. Probably, the best "marker" of afterload is systemic vascular resistance (SVR), also called total peripheral resistance (TPR). However, TPR is not routinely calculated clinically and thus arterial pressure (diastolic, mean, or systolic) is often used as the index of afterload.

Afterload is increased in 3 main situations:

- When aortic pressure is increased (elevated mean arterial pressure); for example, when hypertension increases the afterload, the left ventricle has to work harder to overcome the elevated arterial pressures
- When SVR is increased, resulting in increased resistance and decreased compliance
- In aortic stenosis, resulting in pressure overload of the left ventricle

In general, when afterload increases, there is an initial fall in stroke volume.

VENTRICULAR FUNCTION CURVES

Ventricular function curves are an excellent graphical depiction of the effects of preload versus contractility and afterload.

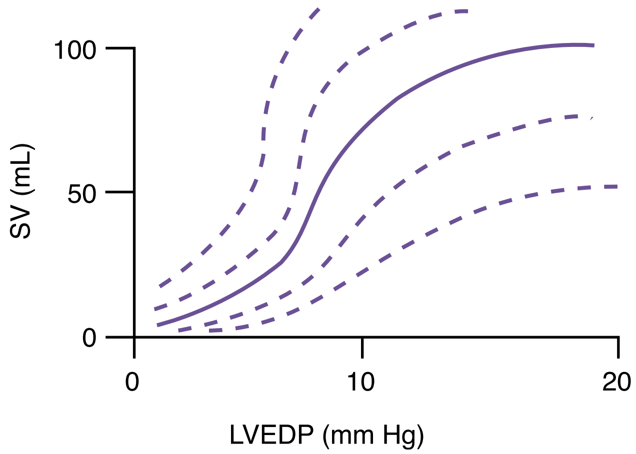


Figure IV-2-3. Family of Frank-Starling Curves

Changes in afterload and contractility shift the curve up or down, left or right.

There is no single Frank-Starling curve on which the ventricle operates. There is actually a family of curves, each of which is defined by the afterload and the inotropic state of the heart:

- Increasing afterload and decreasing contractility shift the curve down and to the right.
- Decreasing afterload and increasing contractility shift the curve up and to the left.

Application of Ventricular Function Curves

A ventricular function curve is the rise in ventricular performance as preload increases (Frank-Starling curve). Thus:

- All points on a ventricular function curve have the same contractility.
- All curves have an ascending limb, a peak point, and possibly a descending limb.
- The pericardium normally prevents the large increases in preload necessary to reach the peak of a cardiac function curve.

y axis: index of systolic performance, e.g., stroke work, stroke volume, cardiac output; all are indices of the force of ventricular contraction

x axis: index of ventricular preload, e.g., ventricular end-diastolic volume or pressure, RAP, or CVP

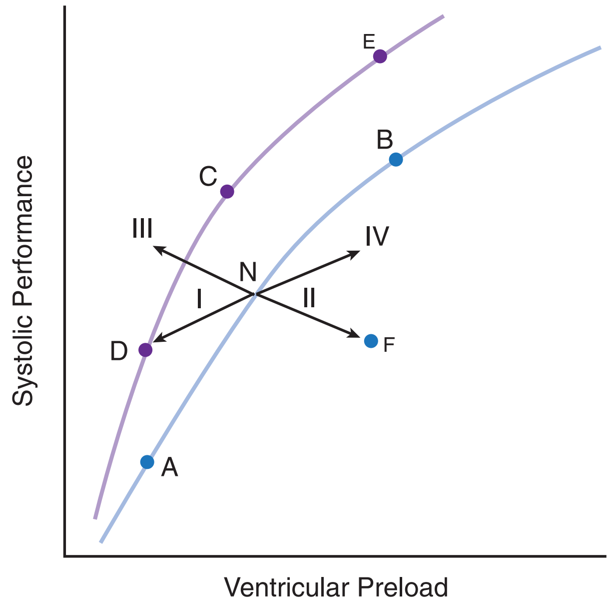


Figure IV-2-4. Ventricular Function Vectors

Starting at N, which represents a normal, resting individual:

- A = decreased performance due to a reduction in preload
- B = increased performance due to an increased preload

Starting at N, point C represents an increased performance due to an increase in contractility or a reduction in afterload.

- Any point above a ventricular function curve means increased contractility, or an acute decrease in afterload.
- Any point below a ventricular function curve means decreased contractility, or an acute increase in afterload.

Points C, D, and E represent different levels of performance due to changes in preload only (Frank-Starling mechanism); all 3 points have the same contractility.

Vector I: consequences of a loss in preload, e.g., hemorrhage, venodilators (nitroglycerin)

- Performance decreases because of a loss in preload.
- The loss of venous return and preload reduces cardiac output and blood pressure, and via the carotid sinus, reflex sympathetic stimulation to the heart increases.
- The increased contractility partially compensates for the loss of preload.
- When there is a loss of either preload or contractility that compromises performance, the other factor usually increases to return performance toward normal. However, the compensatory mechanism is usually incomplete.

Vector II: consequences of a loss in contractility, e.g., congestive heart failure

- Performance decreases because of a loss in contractility.
- A decrease in contractility decreases ejection fraction, which increases preload.
- The increased preload partially compensates for the loss of contractility.
- An acute increase in afterload, e.g., peripheral vasoconstriction, produces the same change.

Vector III: consequences of an acute increase in contractility

- Performance increases.
- The increased contractility increases ejection fraction.
- The increased ejection fraction decreases preload.
- An acute decrease in afterload, e.g., peripheral vasodilation, produces the same shift in the curve.

Vector IV: consequences of an acute increase in preload, e.g., volume loading in the individual going from the upright to the supine position.

- Increased venous return increases preload, which increases performance and cardiac output.
- Increasing cardiac output raises blood pressure, and via the carotid sinus reflex, sympathetic stimulation to the heart decreases.
- The decreased sympathetic stimulation reduces contractility.

All of the preceding sequences assume no dramatic change in heart rate, which could reduce or eliminate some of the expected changes. Whenever there is a change in sympathetic stimulation to the heart, there is a change in both contractility and heart rate.

Ventricular Volumes

End-diastolic volume (EDV): volume of blood in the ventricle at the end of diastole

End-systolic volume (ESV): volume of blood in the ventricle at the end of systole

Stroke volume (SV): volume of blood ejected by the ventricle per beat

$$\mathbf{SV = EDV - ESV}$$

Ejection Fraction (EF): EF = SV/EDV
(should be >55% in a normal heart)

CHRONIC CHANGES: SYSTOLIC AND DIASTOLIC DYSFUNCTION

Systolic dysfunction is an abnormal reduction in ventricular emptying due to impaired contractility or excessive afterload.

Diastolic dysfunction is a decrease in ventricular compliance i.e., the ventricle is stiffer. Reduced compliance causes an elevated diastolic pressure for any given volume. EDV is often reduced, but compensatory mechanisms may result in a normal EDV (although end-diastolic pressure is elevated at this "normal" EDV).

Pressure Overload

- Examples of a pressure overload on the left ventricle include hypertension and aortic stenosis.
- Initially, there is no decrease in cardiac output or an increase in preload since the cardiac function curve shifts to the left (increased performance due to increased contractility).
- Chronically, in an attempt to normalize wall tension (actually internal wall stress), the ventricle develops a concentric hypertrophy. There is a dramatic increase in wall thickness and a decrease in chamber diameter.
- The consequence of concentric hypertrophy (new sarcomeres laid down in parallel, i.e., the myofibril thickens) is a decrease in ventricular compliance and diastolic dysfunction, followed eventually by a systolic dysfunction and ventricular failure.

Volume Overload

- Examples of a volume overload on the left ventricle include mitral and aortic insufficiency and patent ductus arteriosus.
- Fairly well tolerated if developed slowly. A large acute volume overload less well tolerated and can precipitate heart failure.
- Due to the LaPlace relationship, a dilated left ventricle must develop a greater wall tension to produce the same ventricular pressures.

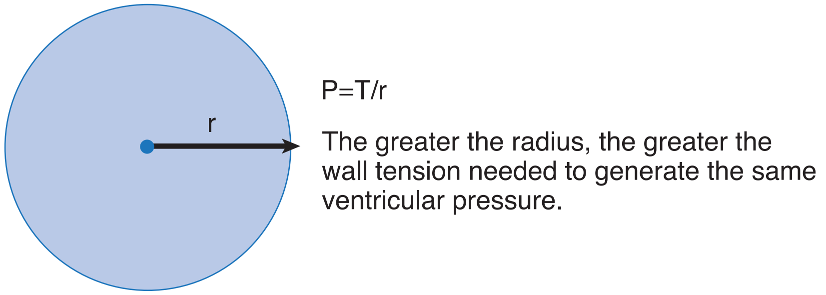


Figure IV-2-5

- Chronically, in an attempt to normalize wall tension (actually external wall stress), the ventricle develops an eccentric hypertrophy (new sarcomeres laid down end-to-end, i.e., the myofibril lengthens).

As cardiac volumes increase, there is a modest increase in wall thickness that does not reduce chamber size.

- Compliance of the ventricle is not compromised and diastolic function is maintained.
- Eventual failure is usually a consequence of systolic dysfunction.

Cardiomyopathy

Cardiac failure or more specifically, congestive failure, is a syndrome with many etiologies. Cardiomyopathy is a failure of the myocardium where the underlying cause originates within the myocyte (excluded would be valvular heart disease, afterload problems, and coronary heart disease).

There are 3 basic types:

- Dilated cardiomyopathy
- Restrictive cardiomyopathy
- Hypertrophic cardiomyopathy

Dilated cardiomyopathy

Dilated cardiomyopathy is ventricular dilation with only a modest hypertrophy that is less than appropriate for the degree of dilation. It can occur for the left heart, right heart, or can include both.

- Diastolic function remains intact and helps compensate for the chamber dilation.
- Compensation also includes increased sympathetic stimulation to the myocardium.
- Systolic dysfunction despite compensations via Frank-Starling and increased contractility
- Further dilation over time and mitral and tricuspid failure enhance systolic dysfunction with eventual complete failure.

Restrictive cardiomyopathy

Restrictive cardiomyopathy is decreased ventricular compliance with diastolic dysfunction and a decrease in ventricular cavity size.

- Increased filling pressures lead to left- and right-sided congestion.
- Ventricular hypertrophy may or may not be present.
- Systolic maintained close to normal

Hypertrophic cardiomyopathy

- Septal or left ventricular hypertrophy is unrelated to a pressure overload.
- Diastolic dysfunction due to increased muscle stiffness and impaired relaxation
- Is a subtype of hypertrophic cardiomyopathy, often resulting in a restriction of the ventricular outflow tract (idiopathic hypertrophic subaortic stenosis) and pulmonary congestion. Currently this is referred to clinically as hypertrophic obstructive cardiomyopathy (HOCM).
- Hypertrophy may be related to septal fiber disarray.

Recall Question

Which of the following physiological changes is characteristic of hypertrophic cardiomyopathy, as opposed to other types of cardiomyopathies?

A. Septal hypertrophy unrelated to pressure overload

B. Decreased ventricular compliance

C. Ventricular dilation with intact diastolic function

D. Systolic dysfunction with mitral valve failure

E. Increased filling pressures leading to left and right sided congestion

Answer: A

CV Regulation and Cardiac Output 3

Learning Objectives

- ❑ Answer questions on short-term regulation of systemic arterial pressure
- ❑ Demonstrate understanding wall tension and the application of the LaPlace relationship

SHORT-TERM REGULATION OF SYSTEMIC ARTERIAL PRESSURE

Arterial Baroreceptors

The baroreceptor reflex is the short-term regulation of blood pressure. Its main features can be seen below.

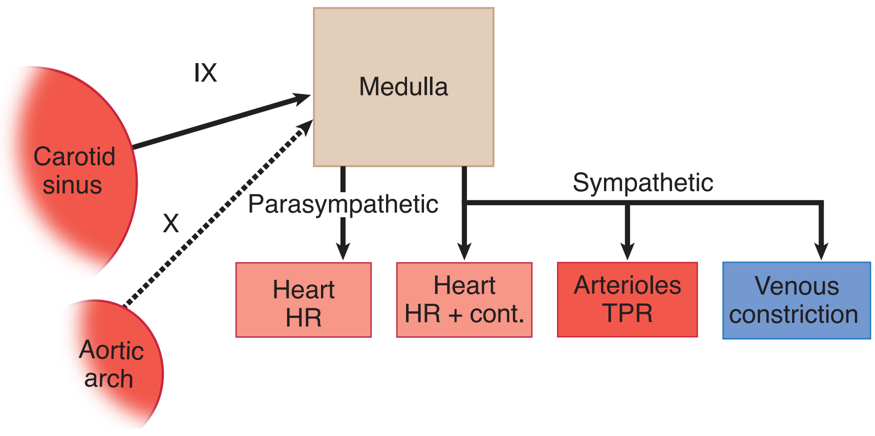


Figure IV-3-1. Baroreflexes

The reninangiotensin-aldosterone system is the long-term regulation of blood pressure.

$$\mathbf{MAP = CO \times TPR}$$

Key points regarding arterial baroreceptors:

- Mechanoreceptors imbedded in the walls of the aortic arch and carotid sinus are stimulated by a rise in intravascular pressure.
- Afferent activity is relayed to the medulla via cranial nerves IX (carotid sinus) and X (aortic arch).

- Baroreceptor activity exists at the person's resting arterial blood pressure.
- Afferent activity stimulates the parasympathetic nervous system and inhibits the sympathetic nervous system.
- A fall in arterial blood pressure evokes a reflex decrease in parasympathetic activity and increase in sympathetic activity. This is a negative feedback system to bring blood pressure back to its original level.
- A rise in arterial blood pressure evokes a reflex increase in parasympathetic activity and fall in sympathetic activity. This is a negative feedback system to bring blood pressure back to its original level.
- Activation of arterial baroreceptors inhibits the secretion of ADH.

Table IV-3-1. Reflex Changes for Specific Maneuvers

Condition	Afferent Activity	Parasympathetic Activity	Sympathetic Activity		
BP increase	↑	↑	↓		
BP decrease	↓	↓	↑	**BP**	**HR**
Carotid occlusion	↓	↓	↑	↑	↑
Carotid massage	↑	↑	↓	↓	↓
Cut afferents	↓	↓	↑	↑	↑
Lying to stand Orthostatic hypotension Fluid loss	↓	↓	↑	↑ toward normal	↑
Volume load Weightlessness	↑	↑	↓	↓ toward normal	↓

Cardiopulmonary Mechanoreceptors (Baroreceptors)

Mechanoreceptors are embedded in the walls of the heart (all 4 chambers), great veins where they empty into the right atrium, and pulmonary artery.

- Afferent activity is relayed to the medulla via cranial nerve X (vagus).
- Because this region is highly compliant, volume changes are the primary stimulus.
- A reduction in volume in the heart and/or the vessels leading to the heart evokes a reflex increase in SNS activity and a decrease in PNS activity.
- A rise in volume in the heart and/or the vessels leading to the heart evokes a reflex decrease in SNS activity and an increase in PNS activity.
- Similar to arterial baroreceptors, this represents a negative feedback regulation of arterial blood pressure. Further, like arterial baroreceptors, activation of these receptors inhibits ADH release.

Application of Hemodynamics to the Systemic Circulation

A simplified model of the circulation can be used to examine whole-body cardiovascular regulation. Blood flows from the aorta to the large arteries that supply the various organs. Within each organ, there are muscular arterioles that serve as the primary site of resistance.

The sum of these resistors (added as reciprocals because of the parallel arrangement) is TPR/SVR. This represents afterload to the heart.

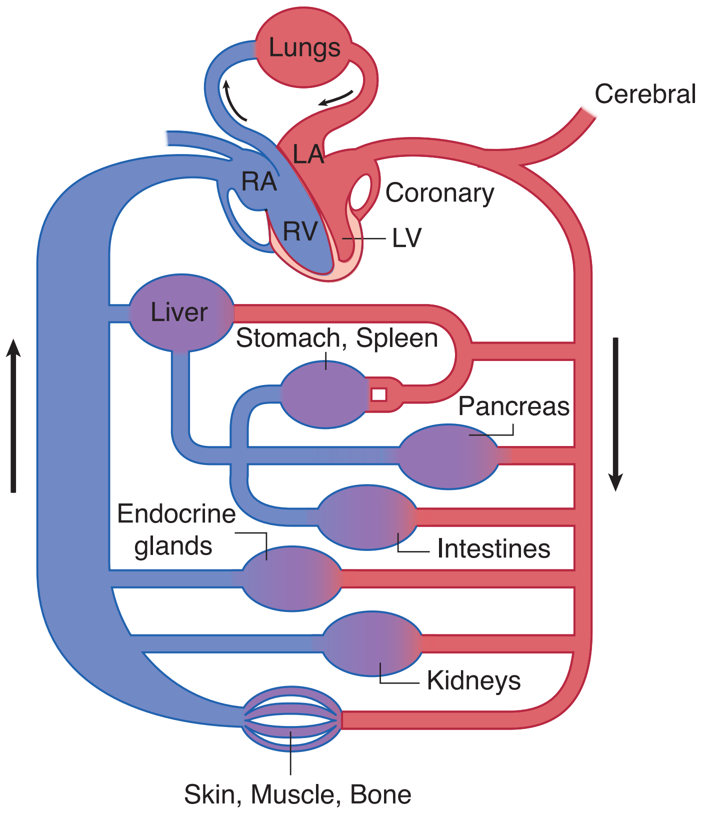


Figure IV-3-2. Systemic Circuit

There are 2 functional consequences related to the fact arterioles serve as the primary site of resistance:

- They regulate blood flow to the capillaries (site of exchange with the tissue).
- They regulate upstream pressure, which is mean arterial pressure (MAP).

Tissues need nutrient delivery and thus have mechanisms to regulate the tone of arterioles (intrinsic regulation, discussed in the next chapter). However, from a whole body perspective it is imperative to maintain an adequate MAP because this is the pressure head (upstream pressure) for the entire body (extrinsic regulation).

Bridge to Pathology

Sepsis, anaphylaxis, and neurogenic shock are examples of uncontrolled vasodilation in the periphery, leading to diminished MAP.

Bridge to Pharmacology

Drugs that mimic NE cause the same cardiovascular effects that NE produces. These include alpha-1 agonists, NE releasers, and NE reuptake inhibitors.

Bridge to Pharmacology

Drugs that block NE's vascular effects (alpha blockers), prevent NE release, liberate NO, activate beta-2 receptors, block calcium entry into smooth muscle cells, and/or open smooth muscle potassium channels mimic the vasodilatory effects indicated.

Given the above, consider arterioles to effectively function as faucets. The tissues need to regulate the faucet to ensure adequate nutrient delivery (intrinsic regulation). On the other hand, these arterioles need a sufficient tone to maintain MAP (extrinsic regulation). If all the faucets were fully opened simultaneously then upstream pressure (MAP) plummets, in turn compromising blood flow to all the organs. Thus, a balance must exist with respect to the level of arteriolar tone ("how tight the faucet is") so there is enough flow to meet the metabolic demands without compromising MAP.

A variety of extrinsic mechanisms exist to regulate arterioles and thus maintain an adequate MAP. Factors that cause vasoconstriction, resulting in increased MAP and reduced flow to the capillary include:

- Norepinephrine (NE) released from sympathetic postganglionic neurons
  - NE binds alpha-1 receptors to activate Gq which increases cytosolic calcium in smooth muscle cells, in turn causing vasoconstriction. The sympathetic nervous system is the dominant regulator of vascular tone and has a tonic effect on skeletal muscle and cutaneous vessels at rest. During times of stress, it can exert its effects on the splanchnic and renal circulations as well.
- Epinephrine (EPI) released from the adrenal medulla also activates alpha-1 receptors.
- Ang II via the AT1 receptor (Gq)
- Arginine vasopressin (AVP), also known as anti-diuretic hormone (ADH), via the V1 receptor (Gq)

Vasodilation of arterioles results in a drop in MAP with an increased flow to capillaries (provided MAP doesn't fall too much). Vasodilatory mechanisms include:

- Decreased sympathetic activity: reduced NE release decreases alpha-1 vasoconstriction
- EPI stimulates vascular beta-2 receptors (Gs–cAMP)
- Nitric oxide (NO): tonically released from vascular endothelium and activates soluble guanylyl cyclase to increase smooth muscle cGMP
- A variety of compounds produced by tissue metabolism, e.g., adenosine, CO_2, K^+, and H^+

VENOUS RETURN

To understand vascular function and thus ultimately the regulation of cardiac output, one can "split" the circulation into 2 components:

- Cardiac output (CO): flow of blood exiting the heart (down arrow on the arterial side).
- Venous return (VR): flow of blood returning to the heart (up arrow on the venous side). Because this is the flow of blood to the heart, it determines preload for the ventricles (assuming normal ventricular function).

Because the circulation is a closed system, these flows are intertwined and must be the same when one examines it "over time" or at steady-state. In addition, each flow is "dependent" on the other. For example:

- If CO fell to zero, then ultimately VR would become zero.
- If one were to stop VR, there would ultimately be no CO.

These are extreme examples to illustrate the point that altering one ultimately alters the other and a variety of factors can transiently or permanently alter each of the variables, resulting in the other variable being impacted to the same degree. Earlier in this book we discussed ventricular function, which plays a pivotal role in CO. In this section, we discuss the regulation of VR. **VR represents vascular function** and thus understanding its regulation sets the stage for understanding CO regulation.

CVP: central venous pressure

IPP: intrapleural pressure

LH: left heart

MABP: mean arterial blood pressure

Psf: mean systemic filling pressure

RH: right heart

RAP: right atrial pressure

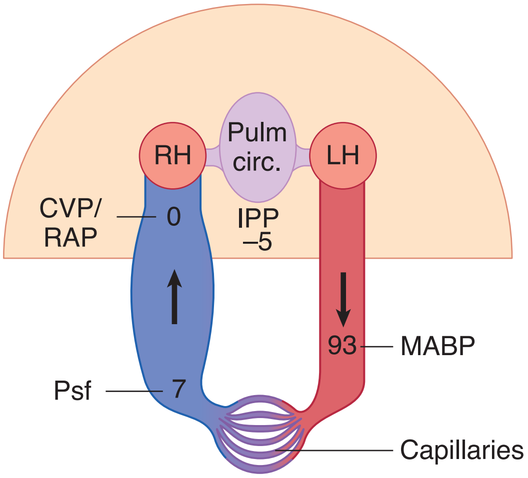


Figure IV-3-3. Pressure Gradients in the Circulatory System

VR is the flow of blood back to the heart and it determines preload. Since it is a flow, it must follow the hemodynamic principles described above, i.e., it is directly proportional to the pressure gradient and inversely related to the resistance.

- **Right atrial pressure (RAP):** blood is flowing to the right atrium, thus RAP is the downstream pressure.
- **Mean systemic filling pressure (Psf):** represents the upstream pressure (pressure head) for VR.

Mean systemic filling pressure (Psf): Although not a "theoretical" pressure (as per numerous experiments, Psf is typically ~7 mm Hg prior to endogenous compensations), this is not a pressure that can be conveniently measured, particularly in a patient. However, because it is the pressure when no flow exists, it is primarily determined by volume and compliance:

- Blood volume: There is a direct relation between blood volume and Psf. The greater the blood volume, the higher the Psf and vice versa.
- Venous compliance: There is an inverse relation between venous compliance and Psf. The more compliant the veins, the lower the Psf and vice versa.

Note

Engaging the muscle pump also increases Psf.

Because Psf is the pressure head (upstream pressure) driving VR, then VR is directly related to Psf. If all other factors are unchanged, it follows that:

- An increase in blood volume increases VR.
- A decrease in blood volume decreases VR.
- A decrease in venous compliance (sympathetic stimulation; muscle pump) increases VR.
- An increase in venous compliance (sympathetic inhibition; venodilators; alpha block) decreases VR.

DETERMINANTS OF CARDIAC OUTPUT

Because VR plays an important role in determining cardiac output (CO), we can now discuss the regulation of CO. The key to remember is that **steady-state CO** is the interplay between **ventricular function** (see ventricular function curves in the previous chapter) and **vascular function**, which is defined by VR curves.

The 4 determinants are as follows:

- Heart rate
- Contractility
- Afterload
- Preload (determined by VR)

The latter 3 factors can be combined on CO/VR curves, which are illustrated and discussed later.

Heart Rate

CO = HR × SV (stroke volume)

Although heart rate (HR) and CO are directly related, the effect of changes in HR on CO is complicated because the other variable, SV, must be considered. High heart rates decrease filling time for the ventricles, and thus can decrease SV. In short, the effect of HR on CO depends upon the cause of the rise in HR.

Endogenously mediated tachycardia, e.g., exercise

In exercise, the rise in HR increases CO. Although filling time is reduced, a variety of changes occur that prevent SV from falling. These are:

- Sympathetic stimulation to the heart increases contractility. This helps maintain stroke volume. In addition, this decreases the systolic interval (see previous chapter) thus preserving some of the diastolic filling time.
- Sympathetic stimulation increases conduction velocity in the heart, thereby increasing the rate of transmission of the electrical impulse.

- Sympathetic stimulation venoconstricts, which helps preserve VR (see above) and ventricular filling.
- The skeletal muscle pump increases VR, helping to maintain ventricular filling.

Pathologically mediated tachycardia, e.g., tachyarrhythmias

- The sudden increase in HR curtails ventricular filling resulting in a fall in CO.
- Although the fall in CO decreases MAP and activates the sympathetic nervous system, this occurs "after the fact" and is thus unable to compensate.
- There is no muscle pump to increase VR.

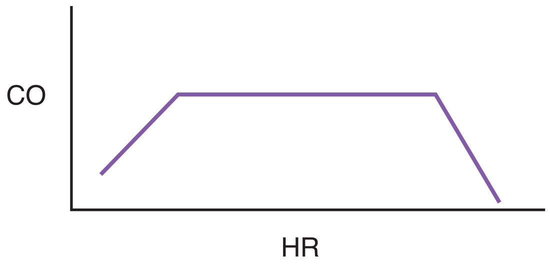


Figure IV-3-4

Contractility

There is a direct relation between contractility and ventricular output. Thus, there is typically a direct relation between contractility and CO.

Afterload

Afterload is the load the heart works against and the best marker of afterload is TPR. There is an inverse relation between afterload and ventricular output, thus there is generally an inverse relation between afterload and CO.

Preload

As discussed earlier, there is a direct relation between preload and ventricular output (Frank-Starling). Presuming there is no change in contractility or afterload, increasing preload increases CO and vice versa.

Cardiac Output/Venous Return Curves

Cardiac output/venous return (CO/VR) curves depict the interplay between ventricular and vascular function indicated in the venous return section above. Steady-state CO is determined by this interplay.

Ventricular function

- X-axis is RAP, a marker of preload.
- Y-axis is CO.
- Thus, this curve is the same as depicted in both figures below, and it defines ventricular function.
- This curve shows that RAP has a positive impact on CO (Frank-Starling mechanism)

Vascular function

- X-axis is RAP, the downstream pressure for VR.
- Y-axis is VR.
- The curve shows that as RAP increases, VR decreases. This is because RAP is the downstream pressure for VR. As RAP increases, the pressure gradient for VR falls, which in turn decreases VR. Thus, RAP has a negative impact on VR.
- X-intercept for the VR curve is Psf (point B on the graph). This is the pressure in the circulation when there is no flow (see section on venous return). Psf is the pressure head (upstream pressure) for VR. Thus, when RAP = Psf, flow (VR) is zero.

Steady-state CO

The intersection of the ventricular and vascular function curves determines steady-state CO (point A in the figure below). In other words, point A represents the interplay between ventricular and vascular function.

- Discounting HR, the only way steady-state CO can change is if ventricular function, or vascular function, or both change.

Solid line: ventricular function

Dashed line: vascular function

A = steady-state cardiac output

All individuals operate at the intersection of the ventricular function and venous return curves.

B = mean systemic filling pressure (Psf)

This is directly related to vascular volume and inversely related to venous compliance.

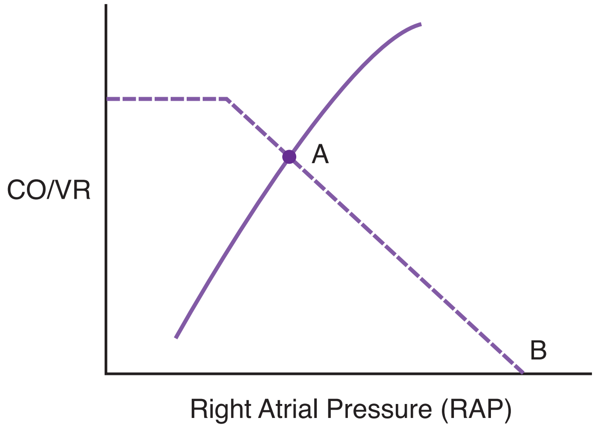


Figure IV-3-5

Resistance

The primary site of resistance for the circulation is the arterioles.

- If arterioles vasodilate (decreased resistance), VR increases (line A of the figure below). Recall that VR is a flow, and thus decreasing resistance increases flow. Note that this vasodilation provides more VR (move up the Frank-Starling curve).

  Although not depicted in the graph, vasodilation decreases afterload and thus shifts the ventricular function curve up and to the left. In short, arteriolar vasodilation enhances both ventricular and vascular function.

- If arterioles vasoconstrict (increased resistance), VR falls (line B of the figure below). Note that this vasoconstriction reduces VR, and steady-state CO falls as one moves down the Frank-Starling curve.

Psf

As indicated above (venous return section), Psf is directly related to blood volume and inversely related to venous compliance.

- Increasing vascular volume (infusion; activation of RAAS) or decreasing venous compliance (sympathetic stimulation; muscle pump; exercise; lying down) increases Psf, causing a right shift in the VR curve (line C of figure below). Thus, either of these changes enhances filling of the ventricles (move up the Frank-Starling curve) and CO.
- Decreasing vascular volume (hemorrhage; burn trauma; vomiting; diarrhea) or increasing venous compliance (inhibit sympathetics; alpha block; venodilators; standing upright) decreases Psf, causing a left shift in the VR curve (line D of figure below). Thus, either of these changes reduces filling of the ventricles (move down the Frank-Starling curve) and CO.

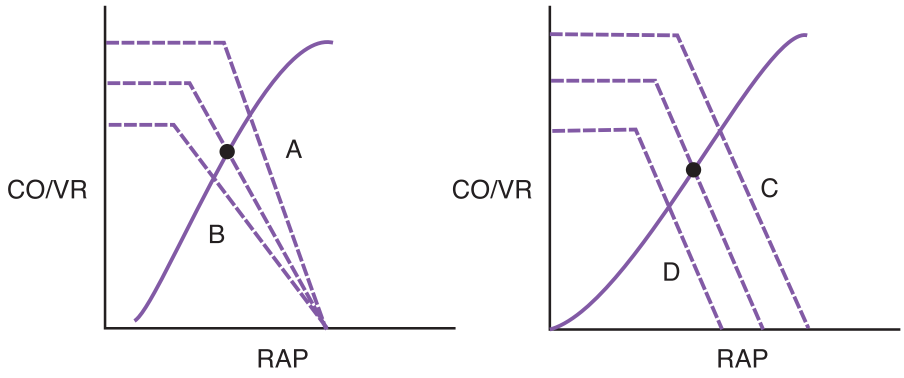


Figure IV-3-6

Although not depicted in the figure, vasoconstriction increases afterload, shifting the ventricular function curve down and to the right. Thus, arteriolar vasoconstriction reduces both ventricular and vascular function.

A: arteriolar dilation

B: arteriolar constriction

C: increased vascular volume; decreased venous compliance

D: decreased vascular volume; increased venous compliance

Solid circles represent starting CO.

Note

The Effect of Gravity

Case 1. When placing a central line in the internal jugular or subclavian vein of a patient in the medical intensive care unit, place the patient in the Trendelenburg position, in which the deep veins of the upper extremity are below the level of the heart. This position makes the venous pressure less negative, thus reducing the risk of forming an "air embolus," in which the needle forms a connection between the positive atmospheric pressure and the negative vein.

Case 2. To take an accurate blood pressure reading, place the sphygmomanometer at the level of the heart. If the cuff is above the level of the heart, the reading will be falsely low; conversely, if the cuff is below the level of the heart, the reading will be falsely high.

Bridge to Pathology/ Pharmacology

The inability to maintain MAP when standing upright is called orthostatic intolerance. In this condition, the fall in MAP reduces cerebral blood flow, causing the patient to feel dizzy or light-headed. This can lead to a syncope event.

One of the more common causes for this is reduced vascular volume. The low volume reduces VR and the added fall in VR (due to venous pooling) overwhelms the compensatory mechanisms. Other factors that can lead to orthostatic intolerance are venodilators, poor ventricular function such as heart failure or cardiac transplant, and dysautonomias.

EFFECT OF GRAVITY

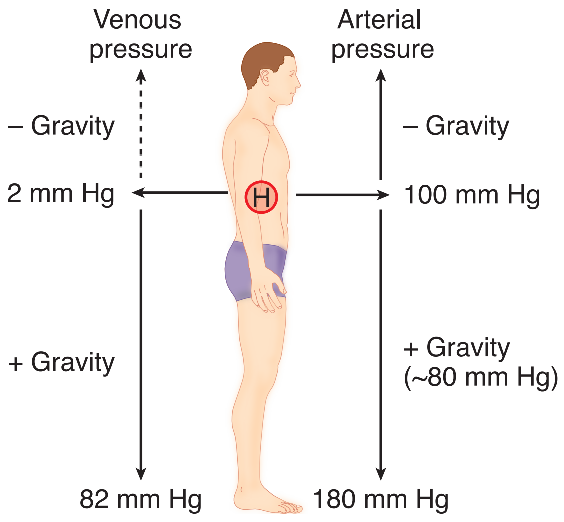


Figure IV-3-7. Effect of Gravity

Below heart level, there are equal increases in systemic arterial and venous pressures (assuming no muscular action). Thus, the pressure difference between arteries and veins does not change.

Because veins are very compliant vessels, the higher pressures in the dependent veins mean a significant pooling of blood, a volume that is not contributing to cardiac output. Although venous compliance doesn't "technically" increase, gravity's impact is functionally the same as an increase in venous compliance.

When a person goes from supine to an upright posture, the following important changes take place:

- Pressure in the dependent veins increases.
- Blood volume in the dependent veins increases.
- VR decreases.
- If no compensations occurred, then MAP would fall because of the diminished SV.

The initial compensation arises from cardiopulmonary mechanoreceptors (described previously in this chapter), which, because their stretch is reduced, activate the SNS and inhibit the PNS.

The reflex activation of the sympathetic nervous system causes:

- Arteriolar vasoconstriction (TPR increases)
- Increase in HR
- Venoconstriction

If MAP falls, then the arterial baroreceptors also participate in the reflex changes.

Above heart level, systemic arterial pressure progressively decreases. Because venous pressure at heart level is close to zero, venous pressure quickly becomes subatmospheric (negative).

Surface veins above the heart cannot maintain a significant pressure below atmospheric and will collapse; however, deep veins and those inside the cranium supported by the tissue can maintain a pressure that is significantly below atmospheric. A consequence of the preceding is that a severed or punctured vein above heart level has the potential for introducing air into the system.

CHARACTERISTICS OF SYSTEMIC ARTERIES

The following figure shows a pressure pulse for a major systemic artery.

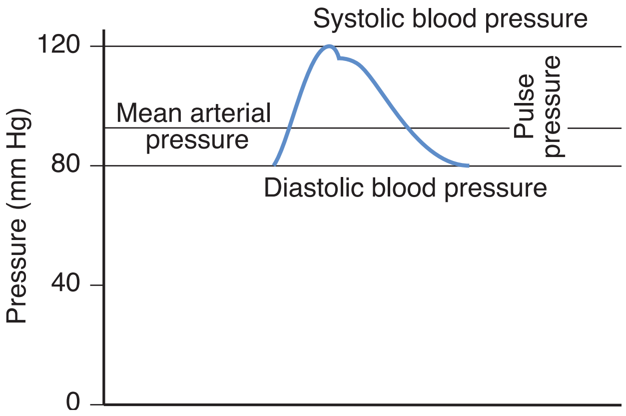


Figure IV-3-8. Pulse Pressure and Mean Pressure

Pulse pressure equals systolic minus diastolic, so here, pulse pressure is 120 – 80 = 40 mm Hg.

Factors Affecting Systolic Pressure

Systolic blood pressure is the highest pressure in the systemic arteries during the cardiac cycle. The main factor determining systolic blood pressure on a beat-to-beat basis is stroke volume.

- An **increase in stroke volume** increases systolic blood pressure, while a **decrease in stroke volume** decreases systolic blood pressure.
- Systolic blood pressure is also directly related to ventricular contractility. In addition, the rate of pressure change in the aorta is directly related to contractility. Thus, if contractility increases, then the rate of pressure and the absolute level of aortic pressure increases, and vice-versa.
- In chronic conditions, a decrease in the compliance of the systemic arteries (age-related arteriosclerosis) also increases systolic blood pressure.

Factors Affecting Diastolic Pressure

Diastolic blood pressure is directly related to the volume of blood left in the aorta at the end of diastole. One important factor determining diastolic blood pressure is TPR.

- **Dilation of the arterioles** decreases diastolic blood pressure, while **constriction of the arterioles** increases diastolic blood pressure.
- HR is the second key factor influencing diastolic pressure and they are directly related: **increased HR increases diastolic blood pressure**, while **decreased HR decreases diastolic blood pressure**.
- Diastolic blood pressure is also directly related to SV, but this is typically not a major factor.

Note

Theoretically, the **systemic pulse pressure** can be conceptualized as being proportional to stroke volume, or the amount of blood ejected from the left ventricle during systole, and inversely proportional to the compliance of the aorta.

Factors Affecting Pulse Pressure

The following increase (widen) pulse pressure:

- An increase in stroke volume (systolic increases more than diastolic)
- A decrease in vessel compliance (systolic increases and diastolic decreases)

The aorta is the most compliant artery in the arterial system. Peripheral arteries are more muscular and less compliant. Based on the preceding information, in the figure below the pressure record on the left best represents the aorta, whereas the one on the right best represents the femoral artery.

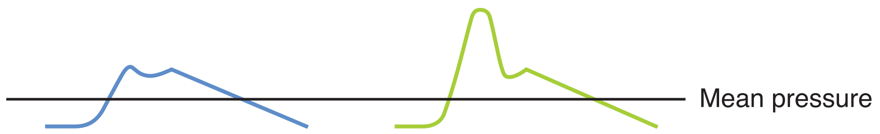


Figure IV-3-9. Compliance and Pulse Pressure

The figure demonstrates that a compliant artery has a small pulse pressure and that a stiff artery has a large pulse pressure. Also, pulse pressure increases with age because compliance is decreasing. This can produce isolated systolic hypertension, in which mean pressure is normal because the elevated systolic pressure is associated with a reduced diastolic pressure.

Factors Affecting Mean Pressure

Mean pressure is pressure averaged over time. It is not the arithmetic mean and is closer to diastolic pressure than to systolic pressure.

Mean pressure can be approximated by the following formulas:

For a blood pressure of 120/80 mm Hg:

$$\text{Mean pressure} = \text{diastolic} + 1/3 \text{ pulse pressure}$$
$$80 + 1/3(40) = 93 \text{ mm Hg}$$
$$= 2/3 \text{ diastolic pressure} + 1/3 \text{ systolic pressure}$$
$$2/3(80) + 1/3(120) = 93 \text{ mm Hg}$$

Any formula that calculates mean pressure must give a value between systolic and diastolic but closer to diastolic than systolic.

The factors affecting mean pressure (application of hemodynamics discussed above) include:

Q: cardiac output
P_1: aortic pressure (mean arterial pressure)
P_2: pressure at the entrance of the right atrium
R: resistance of all vessels in the systemic circuit (referred to as **TPR**)

Because the major component of TPR is the arterioles, TPR can be considered an index of arteriolar resistance.

Because P_1 is a very large number (93 mm Hg) and P_2 is a very small one (~0 mm Hg), that doesn't change dramatically in most situations, we can simplify the equation if we approximate P_2 as zero. Then:

$$\mathrm{CO} = \frac{\mathrm{MAP}}{\mathrm{TPR}} \text{ or } \mathbf{MAP = CO \times TPR}$$

MAP: mean arterial pressure

CO: cardiac output

TPR: total peripheral resistance

This equation simply states that:

- MAP is determined by only 2 variables: cardiac output and TPR.
- CO is the circulating volume. The blood stored in the systemic veins and the pulmonary circuit would not be included in this volume.
- TPR is the resistance of all vessels in the systemic circuit. By far the largest component is the resistance in the arterioles.
- However, if venous or right atrial pressure (RAP) is severely increased, it must be taken into account when estimating TPR. In this case, the formula is:

$$\mathbf{(MAP - RAP) = CO \times TPR}$$

or rearranged to solve for resistance: $\mathrm{TPR} = \dfrac{(\mathrm{MAP} - \mathrm{RAP})}{\mathrm{CO}}$

Recall Question

Which of the following is accurate regarding mean systemic filling pressure (Psf)?

A. IV fluid infusion decreases mean systemic filling pressure

B. Exercising decreases mean systemic filling pressure

C. The volume of blood and the mean systemic filling pressure are proportional

D. Venous compliance and mean systemic filling pressure are directly related

E. Decreasing vascular volume causes mean systemic filling pressure to increase

Answer: C

Regulation of Blood Flow 4

Learning Objectives

- ❏ Demonstrate understanding of Fick principle of blood flow
- ❏ Interpret scenarios on blood flow regulation
- ❏ Explain information related to blood flow to the various organs
- ❏ Demonstrate understanding of fetal circulation
- ❏ Explain information related to cardiovascular stress: exercise

FICK PRINCIPLE OF BLOOD FLOW

The Fick principle can be utilized to calculate the blood flow through an organ. Calculation of flow through the pulmonary circuit provides a measure of the cardiac output (CO).

$$\textbf{Flow} = \frac{\textbf{uptake}}{\textbf{A} - \textbf{V}}$$

Required data are: oxygen consumption of the organ

A – V oxygen content (concentration) difference across organ (not PO_2)

Pulmonary venous (systemic arterial)

oxygen content = 20 vol%
= 20 volumes O_2 per 100 volumes blood
= 20 mL O_2 per 100 mL blood
= 0.2 mL O_2 per mL blood

If pulmonary vessel data are not available, you may substitute arterial oxygen content for pulmonary venous blood and use venous oxygen content in place of pulmonary artery values.

In a normal resting individual, that would appear as follows:

Note

The **Fick principle** was first devised as a technique for measuring CO. It is a way to calculate oxygen consumption (VO_2).

$$VO_2 = CO \times (CaO_2 - CvO_2)$$

CaO_2 = total arterial oxygen content

$(Hgb \times 1.36 \times SaO_2) + PaO_2 \times 0.0031$

These values are obtained from an ABG.

CvO_2 = total venous oxygen content

$(Hgb \times 1.36 \times SvO_2) + PvO_2 \times 0.0031$

These values are obtained from a central venous or Swan-Ganz catheter, which samples blood from the pulmonary artery.

The $(CaO_2 - CvO_2)$ and CO are the 2 main factors that allow variation in the body's total oxygen consumption.

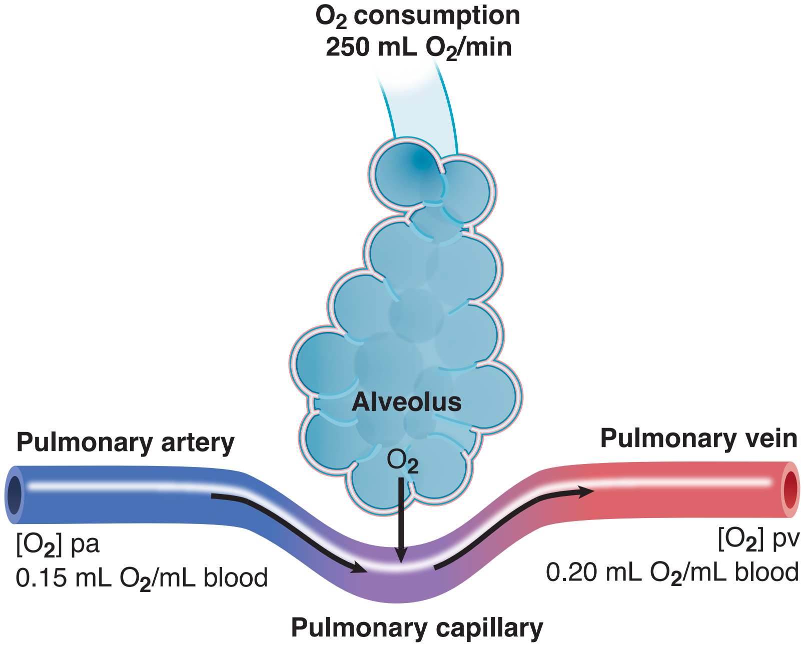


Figure IV-4-1. Alveolar Oxygen Uptake

$$Q(\text{flow}) = \frac{\text{oxygen consumption}}{[O_2]\text{pv} - [O_2]\text{pa}}$$

$$= \frac{250 \text{ mL / min}}{0.20 \text{ mL / mL} - 0.15 \text{ mL / mL}} = 5{,}000 \text{ mL / min}$$

$$\text{Cardiac index} = \frac{\text{cardiac output}}{\text{body surface area}}$$

This would normalize the value for body size.

Application of the Fick Principle

Rearranging the Fick Principle to O_2 consumption = Q × (CaO_2 − CvO_2) can be applied to important concepts regarding homeostatic mechanisms and pathologic alterations. CaO_2 − CvO_2 represents the extraction of O_2 by the tissue.

O_2 consumption

O_2 consumption is dependent upon flow and the extraction of O_2. If tissue O_2 consumption increases, then flow or extraction or both must increase.

- The rise in flow in response to a rise in tissue O_2 consumption is the result of increased production of vasodilator metabolites (see metabolic mechanism below).
- In short, this change in flow and extraction represents homeostatic mechanisms designed to ensure adequate O_2 availability and thus sufficient ATP production.

O_2 delivery

The "first part" of the Fick Principle indicates that delivery of O_2 to the tissue is dependent upon Q and the total amount of O_2 in the blood (CaO_2).

$$O_2 \text{ delivery} = Q \times CaO_2$$

- For any given tissue O_2 consumption, reduced delivery of O_2 results in increased lactic acid production and possible hypoxic/ischemic damage to tissues.
- For any given tissue O_2 consumption, if O_2 delivery decreases, then PvO_2 and SvO_2% fall.

Clinical application: A fall in PvO_2 or SvO_2% indicates the patient's O_2 consumption increased and/or there was a fall in Q or CaO_2 or both.

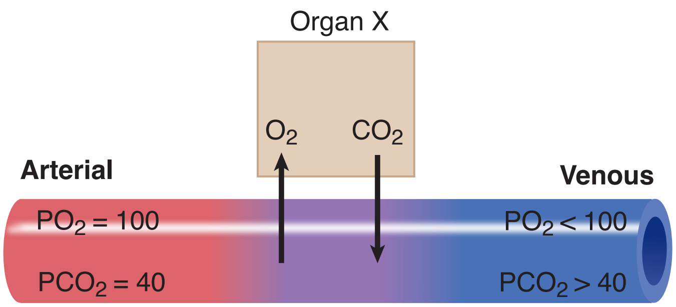


Figure IV-4-2. Application of the Fick Principle

BLOOD FLOW REGULATION

Flow is regulated by constricting and dilating the smooth muscle surrounding the arterioles.

Intrinsic Regulation (Autoregulation)

The control mechanisms regulating the arteriolar smooth muscle are entirely within the organ itself.

- What is regulated is blood flow, not resistance. It is more correct to say that resistance is changed in order to regulate flow.
- No nerves or circulating substances are involved in autoregulation. Thus, the autonomic nervous system and circulating epinephrine have nothing to do with autoregulation.

There are 2 main mechanisms which explain autoregulation.

Metabolic mechanism

- Tissue produces a vasodilatory metabolite that regulates flow, e.g., adenosine, CO_2, H^+, and K^+.
- A dilation of the arterioles is produced when the concentration of these metabolites increases in the tissue. The arterioles constrict if the tissue concentration decreases.

Myogenic mechanism

- Increased perfusing pressure causes stretch of the arteriolar wall and the surrounding smooth muscle.
- Because an inherent property of the smooth muscle is to contract when stretched, the arteriole radius decreases, and flow does not increase significantly.

Major characteristics of an autoregulating tissue

Blood flow should be independent of blood pressure.

This phenomenon is demonstrated for a theoretically perfect autoregulating tissue. The range of pressure over which flow remains nearly constant is the **autoregulatory range**.

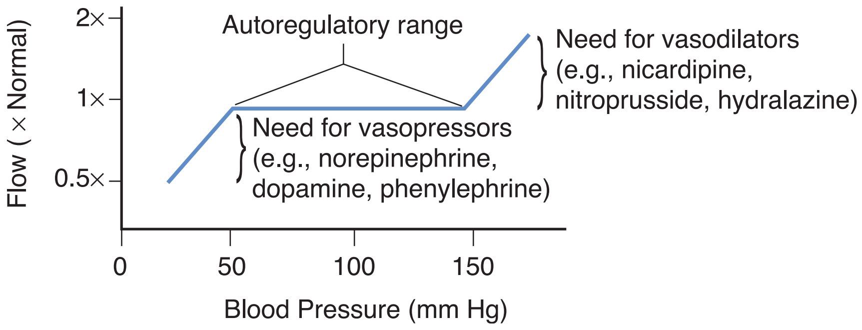


Figure IV-4-3. Autoregulation

Blood flow in most cases is proportional to tissue metabolism. Blood flow is independent of nervous reflexes (e.g., carotid sinus) or circulating humoral factors.

Autoregulating tissues include (tissues least affected by nervous reflexes):

- Cerebral circulation
- Coronary circulation
- Skeletal muscle vasculature during exercise

Extrinsic Regulation

These tissues are controlled by nervous and humoral factors originating outside the organ, e.g., resting skeletal muscle. Extrinsic mechanisms were covered earlier in the book.

The figure below illustrates an arteriole in skeletal muscle and the factors regulating flow under resting conditions.

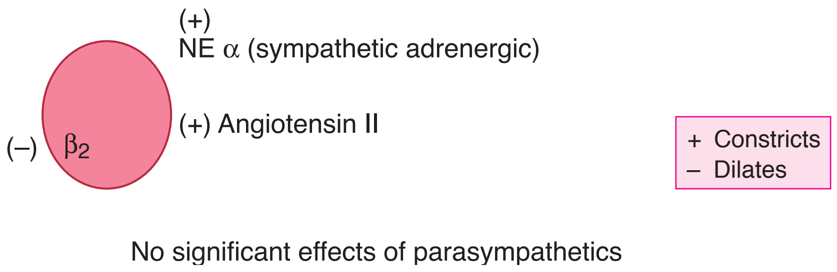


Figure IV-4-4. Resting Skeletal Muscle Blood Flow

The key points for extrinsic regulation are:

- Norepinephrine (NE) released from sympathetic nerves has a tonic influence on arteriolar tone (α receptors) in resting skeletal muscle and skin vasculature in a thermo-neutral environment.
- In times of stress, sympathetic activation can evoke substantial vasoconstriction in the aforementioned tissues, but can also greatly affect renal and splanchnic circulations.
- Epinephrine can evoke vasodilation by binding to vascular β2 receptors.
- With the exception of the penis, the parasympathetic nervous system does not affect arteriolar tone.

Control of Resting versus Exercising Muscle

Resting muscle

Flow is controlled mainly by increasing or decreasing sympathetic α-adrenergic activity.

Exercising muscle

The elevated metabolism in exercising skeletal muscle demands an increase in blood flow (see application of the Fick principle above). In addition, the increased tissue O_2 consumption results in a fall in the PvO_2 of blood leaving the working muscle. The primary mechanisms for increasing flow are:

- Production of vasodilator metabolites, e.g., adenosine, CO_2, H^+, and K^+ causes marked vasodilation. In addition, these metabolites diminish NE's ability to vasoconstrict the arterioles. Further, the increased endothelial shear-stress of the high flow liberates NO.
- Muscle pump

BLOOD FLOW TO THE VARIOUS ORGANS

Coronary Circulation

Coronary flow patterns

Characteristics of left coronary flow (flow to the left ventricular myocardium):

Note

Use caution with drugs such as dobutamine, which can increase contractility through β_1 receptors but can also cause hypotension with some β_2 activation.

Left ventricular contraction causes severe mechanical compression of subendocardial vessels. Therefore:

- Very little if any blood flow occurs during systole.
- Most of the blood flow is during diastole.
- Some subepicardial flow occurs during systole.

Characteristics of right coronary blood flow (flow to the right ventricular myocardium):

Right ventricular contraction causes modest mechanical compression of intramyocardial vessels. Therefore:

- Significant flow can occur during systole.
- The greatest flow under normal conditions is still during diastole.

Oxygenation

In the coronary circulation, the tissues extract almost all the oxygen they can from the blood, even under "basal" conditions. Therefore:

- The venous PO_2 is extremely low. It is the lowest venous PO_2 in a resting individual.
- Because the extraction of oxygen is almost maximal under resting conditions, increased oxygen delivery to the tissue can be accomplished only by increasing blood flow (Fick principle).
- In the coronary circulation, flow must match metabolism.
- Coronary blood flow is most closely related to cardiac tissue oxygen consumption and demand.

Pumping action

Coronary blood flow (mL/min) is determined by the pumping action, or **stroke work** times heart rate, of the heart. Increased pumping action means increased metabolism, which increases the production of vasodilatory metabolites. In turn, coronary flow increases.

Increased pump function occurs with the following:

- An increase in any of the parameters which determine CO: **HR**, **contractility**, **afterload**, **preload**
- HR, contractility, and afterload (often called pressure work) are more metabolically costly than the work associated with preload (volume work).
- Thus, conditions in which HR, contractility, and/or afterload increase, e.g., hypertension, aortic stenosis, and exercise require a greater increase in flow compared to conditions that only increase volume work (supine, aortic regurgitation, volume loading).

Cerebral Circulation

Flow is proportional to arterial PCO_2. Under normal conditions, arterial PCO_2 is an important factor regulating cerebral blood flow.

- Hypoventilation increases arterial PCO_2, thus it increases cerebral blood flow.
- Hyperventilation decreases arterial PCO_2, thus it decreases cerebral blood flow.

As long as arterial PO_2 is normal or above normal, cerebral blood flow is regulated via arterial PCO_2.Therefore:

- If a normal person switches from breathing room air to 100% oxygen, there is no significant change in cerebral blood flow.
- However, a (large) decrease in arterial PO_2 increases cerebral blood flow; an example is high-altitude pulmonary edema (HAPE). Under these conditions, it is the low arterial PO_2 that is determining flow.
- Baroreceptor reflexes do not affect flow.

Intracranial pressure is an important pathophysiologic factor that can affect cerebral blood flow.

Cutaneous Circulation

Cutaneous circulation is almost entirely controlled via the sympathetic adrenergic nerves.

- Large venous plexus innervated by sympathetics
- A-V shunts innervated by sympathetics
- Sympathetic stimulation to the skin causes:
  - Constriction of arterioles and a decrease in blood flow, which is one reason why physicians use a central line to administer vasopressors to prevent distal necrosis
  - Constriction of the venous plexus and a decrease in blood volume in the skin
- Sympathetic activity to the skin varies mainly with the body's need for heat exchange with the environment.

Increased skin temperature directly causes vasodilation, which increases heat loss.

Temperature regulation

There are temperature-sensitive neurons in the anterior hypothalamus, whose firing rate reflects the temperature of the regional blood supply.

- Normal set point: oral 37°C (rectal + 0.5°C)
- Circadian rhythm: low point, morning; high point, evening

The body does not lose the ability to regulate body temperature during a fever. It simply regulates body temperature at a higher set point.

Bridge to Anatomy

The **splanchnic circulation** is composed of the gastric small intestinal, colonic, pancreatic, hepatic, and splenic circulations, arranged in parallel with one another. The three major arteries that supply the splanchnic organs are the celiac, superior, and inferior mesenteric arteries.

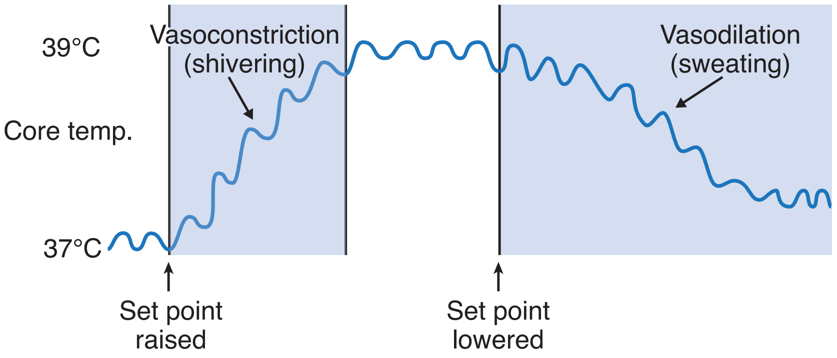


Figure IV-4-5. Temperature Regulation

When a fever develops, body temperature rises toward the new higher set point. Under these conditions, heat-conserving and heat-generating mechanisms include:

- Shivering
- Cutaneous vasoconstriction

After a fever "breaks," the set point has returned to normal, and body temperature is decreasing. Heat-dissipating mechanisms include:

- Sweating (sympathetic cholinergics)
- Cutaneous vasodilation

Renal and Splanchnic Circulation

A small change in blood pressure invokes an autoregulatory response to maintain renal and splanchnic blood flows. Thus, under normal conditions, the renal and splanchnic circulations demonstrate autoregulation.

- Situations in which there is a large increase in sympathetic activity (e.g., hypotension) usually cause vasoconstriction and a decrease in blood flow.
- Renal circulation is greatly overperfused in terms of nutrient requirements, thus the venous PO_2 is high.
- About 25% of the CO goes to the splanchnic circulation, thus it represents an important reservoir of blood in times of stress.
- Splanchnic blood flow increases dramatically when digesting a meal.

Pulmonary Circuit

Characteristics

- Low-pressure circuit, arterial = 15 mm Hg, venous = 5 mm Hg; small pressure drop indicates a low resistance.
- High flow, receives entire CO
- Very compliant circuit; both arteries and veins are compliant vessels

- Hypoxic vasoconstriction (low alveolar PO_2 causes local arteriolar vasoconstriction)
- Blood volume proportional to blood flow: due to the very compliant nature of the pulmonary circuit, large changes in output of the right ventricle are associated with only small changes in pulmonary pressures

Pulmonary response to exercise

- A large increase in cardiac output means increased volume pumped into the circuit. This increases pulmonary intravascular pressures.
- Because of the compliant nature of the circuit, the pulmonary arterial system distends.
- In addition, there is recruitment of previously unperfused capillaries. Because of this recruitment and distension, the overall response is a large decrease in pulmonary vascular resistance (PVR).
- Consequently, when CO is high, e.g., during exercise, there is only a slight increase in pulmonary pressures.
  - Without this recruitment and distension, increasing CO would result in a very high pulmonary artery pressure.

Pulmonary response to hemorrhage

- A large decrease in CO reduces intravascular pulmonary pressures.
- Because these vessels have some elasticity, pulmonary vessels recoil. In addition, there is derecruitment of pulmonary capillaries, both of which contribute to a rise in PVR.
- Consequently, during hemorrhage, there is often only a slight decrease in pulmonary artery pressure.
- Vessel recoil also means less blood is stored in this circuit.

FETAL CIRCULATION

The general features of the fetal circulatory system are shown below. The bolded numbers refer to the percent hemoglobin (%HbO_2) saturation.

- Of the fetal CO, 55% goes to the placenta.
- The umbilical vein and ductus venosus have highest %HbO_2 saturation (80%).
- When mixed with inferior vena caval blood (26% HbO_2), the %HbO_2 saturation of blood entering the right atrium is 67%.
- This blood is directed through the foramen ovale to the left atrium, left ventricle, and ascending aorta to perfuse the head and the forelimbs.
- Superior vena caval blood (40% HbO_2) is directed through the tricuspid valve into the right ventricle and pulmonary artery and shunted by the ductus arteriosus to the descending aorta. Shunting occurs because fetal pulmonary vascular resistance is very high, so 90% of the right ventricular output flows into the ductus arteriosus and only 10% to the lungs.
- The percent HbO_2 saturation of aortic blood is 60%.

- Fifty-five percent of the fetal CO goes through the placenta. At birth, the loss of the placental circulation increases systemic resistance. The subsequent rise in aortic blood pressure (as well as the fall in pulmonary arterial pressure caused by the expansion of the lungs) causes a reversal of flow in the ductus arteriosus, which leads to a large enough increase in left atrial pressure to close the foramen ovale.

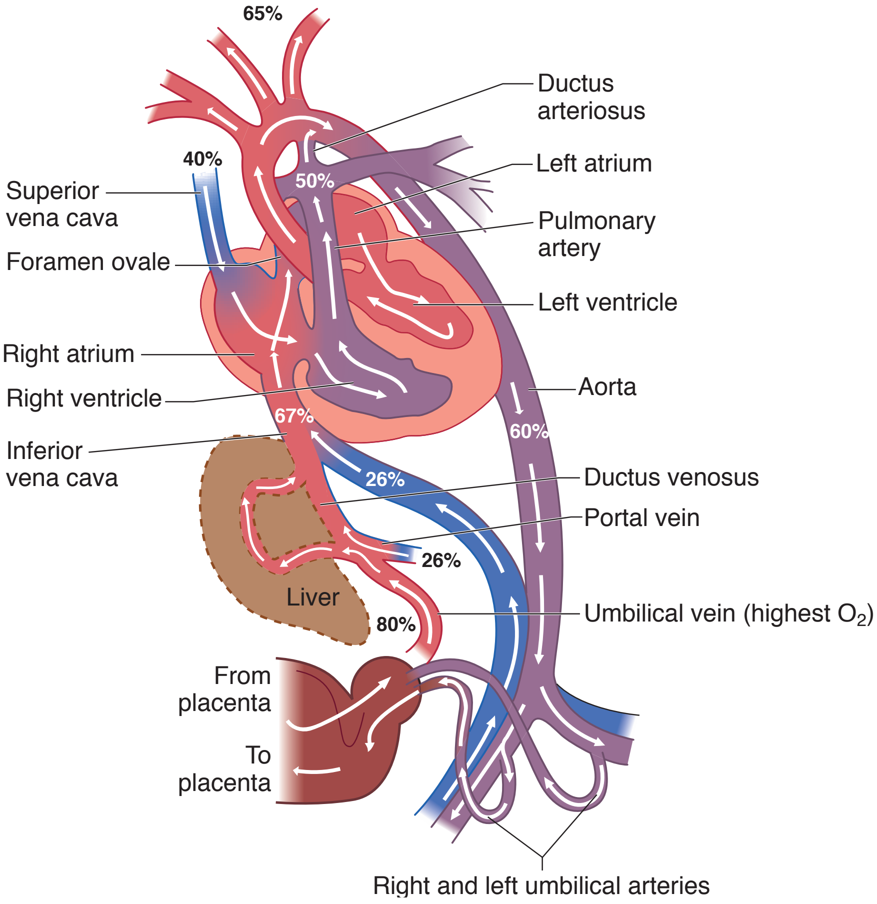


Figure IV-4-6. Fetal Circulatory System

Recall Question

Which of the following regulates cerebral blood flow in a patient suffering from high-altitude pulmonary edema?

A. Arterial PO_2

B. Arterial PCO_2

C. Arterial HCO^3

D. Arterial H^+

E. Cerebral PO_2

Answer: A

CARDIOVASCULAR STRESS: EXERCISE

The following assumes the person is in a steady state, performing moderate exercise at sea level.

Pulmonary Circuit

- Blood flow (CO): large increase
- Pulmonary arterial pressure: slight increase
- Pulmonary vascular resistance: large decrease
- Pulmonary blood volume: increase
- Number of perfused capillaries: increase
- Capillary surface area: increase, i.e., increased rate of gas exchange

Systemic Circuit

Arterial system

- PO_2: no significant change, hemoglobin still fully saturated
- PCO_2: until one approaches maximal O_2 consumption, there is no significant change; thus the increase in ventilation is proportional to the increase in metabolism
- pH: no change or a decrease due mainly to the production of lactic acid
- Mean arterial pressure: slight increase
- Body temperature: slight increase
- Vascular resistance (TPR): large decrease, dilation of skeletal muscle beds

Venous system

- PO_2: decrease
- PCO_2: increase

Regional Circulations

Exercising skeletal muscle

- Vascular resistance decreases.
- Blood flow increases.
- Capillary pressure increases.
- Capillary filtration increases.
- Lymph flow increases.
- As predicted by the Fick principle, oxygen extraction increases and venous PO_2 falls.

Cutaneous blood flow

Initial decrease, then an increase to dissipate heat

Coronary blood flow

Increase due to increased work of the heart

Cerebral blood flow

No significant change (arterial CO_2 remains unchanged)

Renal and GI blood flow

Both decrease

Physical conditioning

- Regular exercise increases maximal oxygen consumption ($\dot{V}O_2$max) by:
  - Increasing the ability to deliver oxygen to the active muscles. It does this by increasing the CO.
  - The resting conditioned heart has a lower heart rate but greater stroke volume (SV) than does the resting unconditioned heart.
  - At any level of exercise, stroke volume is elevated.
  - However, the maximal heart rate remains similar to that of untrained individuals.
- Regular exercise also increases the ability of muscles to utilize oxygen. There are:
  - An increased number of arterioles, which decreases resistance during exercise.
  - An increased capillary density, which increases the surface area and decreases diffusion distance.
  - An increased number of oxidative enzymes in the mitochondria.

5 Cardiac Cycle and Valvular Heart Disease

Learning Objectives

- ❏ Interpret scenarios on normal cardiac cycle
- ❏ Interpret scenarios on pressure-volume loops
- ❏ Interpret scenarios on valvular dysfunction

NORMAL CARDIAC CYCLE

The figure below illustrates the most important features of the cardiac cycle.

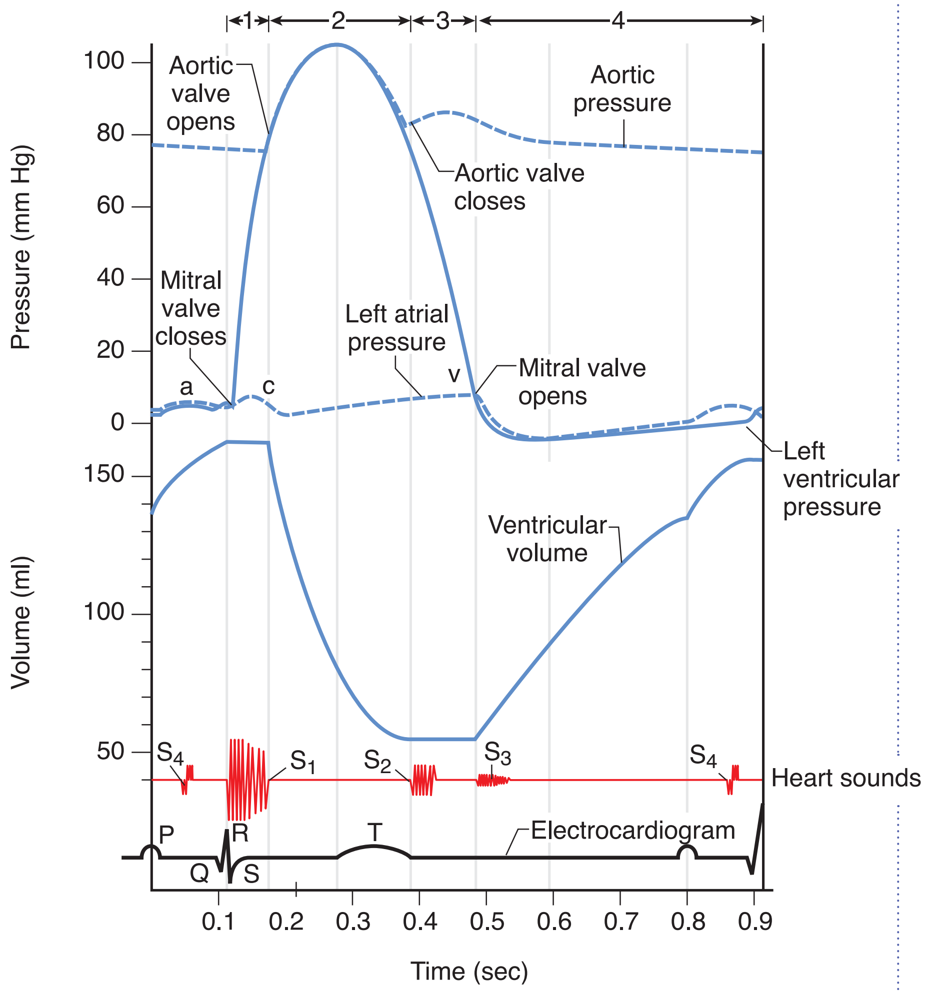


Figure IV-5-1. Cardiac Cycle

Note the most important aspects:

- → QRS → contraction of ventricle → rise in ventricular pressure above atrial pressure → closure of mitral valve
- It is always a pressure difference that causes the valves to open or close.
- Closure of the mitral valve terminates the ventricular filling phase and begins iso-volumetric contraction.
- Isovolumetric contraction: no change in ventricular volume, and both valves (mitral, aortic) closed; ventricular pressure increases and volume is equivalent to end-diastolic volume
- Opening of the aortic valve terminates isovolumetric contraction and begins the ejection phase. The aortic valve opens because pressure in the ventricle slightly exceeds aortic pressure.
- Ejection phase: ventricular volume decreases, but most rapidly in early stages; ventricular and aortic pressures increase initially but decrease later in phase
- Closure of the aortic valve terminates the ejection phase and begins isovolumetric relaxation. The aortic valve closes because pressure in the ventricle goes below aortic pressure. Closure of the aortic valve creates the dicrotic notch.
- Isovolumetric relaxation: no change in ventricular volume and both valves (mitral, aortic) closed; ventricular pressure decreases and volume is equivalent to end-systolic volume
- Opening of the mitral valve terminates isovolumetric relaxation and begins the filling phase. The mitral valve opens because pressure in the ventricle goes below atrial pressure.
- Filling phase: the final relaxation of the ventricle occurs after the mitral valve opens and produces a rapid early filling of the ventricle; this rapid inflow will in some cases induce the third heart sound.
  - The final increase in ventricular volume is due to atrial contraction, which is responsible for the fourth heart sound.
- In a young, healthy individual, atrial contraction doesn't provide significant filling of the ventricle. However, the contribution of atrial contraction becomes more important when ventricular compliance is reduced.

Heart Sounds

The systolic sounds are due to the sudden closure of the heart valves. Normally the valves on the left side of the heart close first. Valves on the right side open first.

Systolic sounds

S1: produced by the closure of the mitral and tricuspid valves

- Valves close with only a separation of about 0.01 seconds which the human ear can appreciate only as a single sound

S2: produced by the closure of the aortic (A2 component) and pulmonic valves (P2 component)

- Heard as a single sound during expiration but during inspiration the increased output of the right heart causes a physiological splitting

The figure below illustrates several situations where splitting of the second heart sound may become audible.

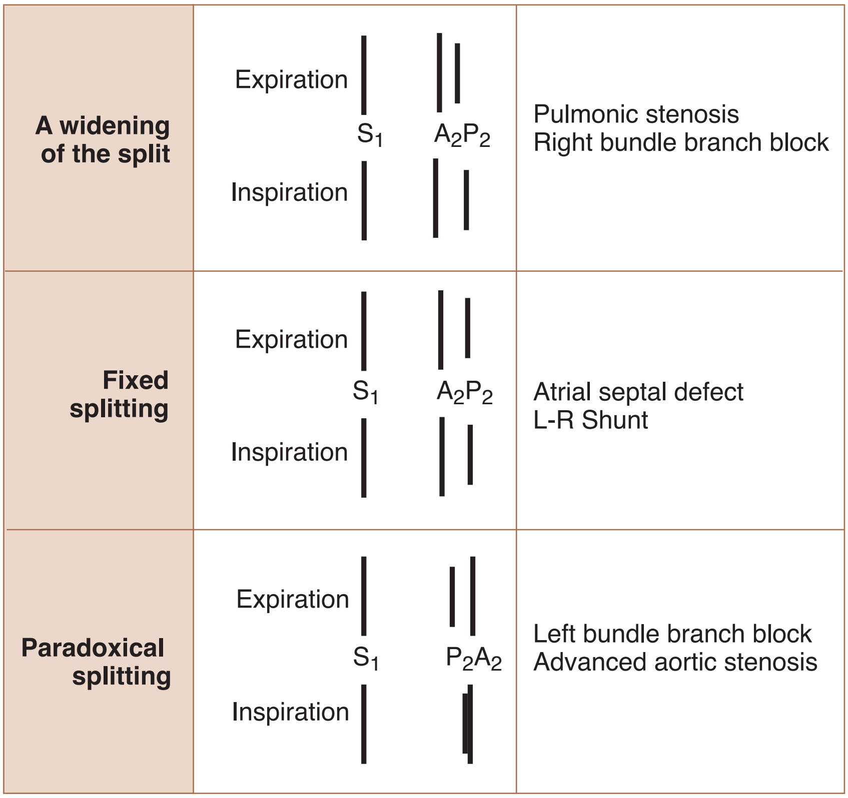


Figure IV-5-2. Abnormal Splitting of the Second Heart Sound (S_2)

S3: when it is present, occurs just after the opening of the AV valves during the rapid filling of the ventricle

- Tends to be produced by rapid expansion of a very compliant ventricle
- In children and young adults, is a normal finding
- In older adults, it occurs with volume overload and is often a sign of cardiac disease

S4: coincident with atrial contraction and is produced when the atrium contracts against a stiff ventricle

- Examples include concentric hypertrophy, aortic stenosis, and myocardial infarction

Venous Pulse

The jugular pulse is generated by changes on the right side of the heart. The pressures will generally vary with the respiratory cycle and are typically read at the end of expiration when intrapleural pressure is at its closest point to zero.

A normal jugular venous pulse tracing can be seen below.

Clinical Correlate

Site of auscultation points:

- Aortic: Second intercostal space on the right side, about mid-clavicular line
- Pulmonic: Second intercostal space on the left side, about mid-clavicular line
- Tricuspid: Fifth intercostal space, just at the left sternal border
- Mitral: Sixth intercostal space on the left side, about mid-clavicular line

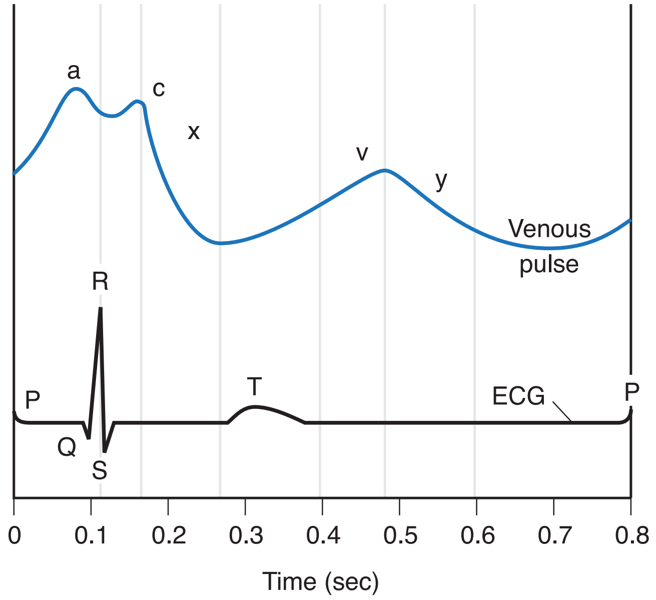


Figure IV-5-3. Venous Pulse and the ECG

a wave

- Highest deflection of the venous pulse and produced by the contraction of the right atrium
- Correlates with the PR interval
- Is prominent in a stiff ventricle, pulmonic stenosis, and insufficiency
- Is absent in atrial fibrillation

c wave

- Mainly due to the bulging of the tricuspid valve into the atrium (rise in right atrial pressure)
- Occurs near the beginning of ventricular contraction (is coincident with right ventricular isovolumic contraction)
- Is often not seen during the recording of the venous pulse

x descent

- Produced by a decreasing atrial pressure during atrial relaxation
- Separated into two segments when the c wave is recorded
- Alterations occur with atrial fibrillation and tricuspid insufficiency

v wave

- Produced by the filling of the atrium during ventricular systole when the tricuspid valve is closed
- Corresponds to T wave of the EKG
- A prominent v wave would occur in tricuspid insufficiency and right heart failure

y descent

- Produced by the rapid emptying of the right atrium immediately after the opening of the tricuspid valve
- A more prominent wave in tricuspid insufficiency and a blunted wave in tricuspid stenosis.

Some abnormal venous pulses are shown below.

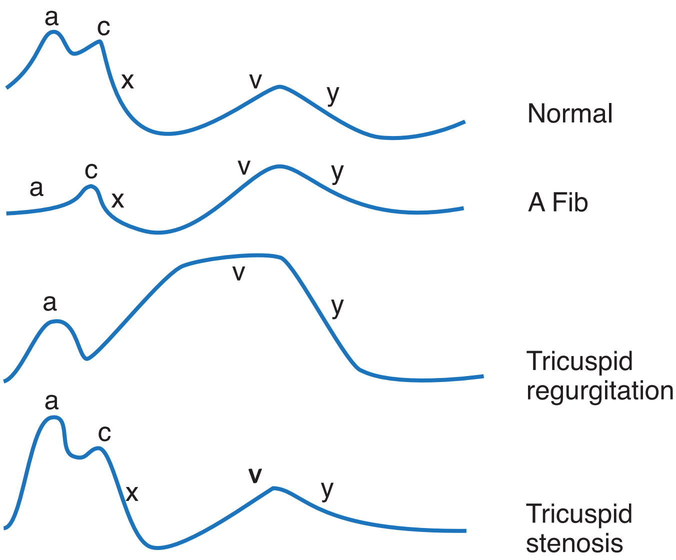


Figure IV-5-4. Normal Versus Abnormal Jugular Pulses

Similar recordings to the systemic venous pulse are obtained when recording pulmonary capillary wedge pressure. Left atrium mechanical events are transmitted in a retrograde manner, although they are somewhat damped and delayed.

The figure below shows the pressure recording from the tip of a Swan-Ganz catheter inserted through a systemic vein through the right side of the heart into the pulmonary circulation and finally with the tip wedged in a small pulmonary artery. The pressure recorded at the tip of the catheter is referred to as pulmonary capillary wedge pressure and is close to left atrial pressure and is an index of preload on the left ventricle.

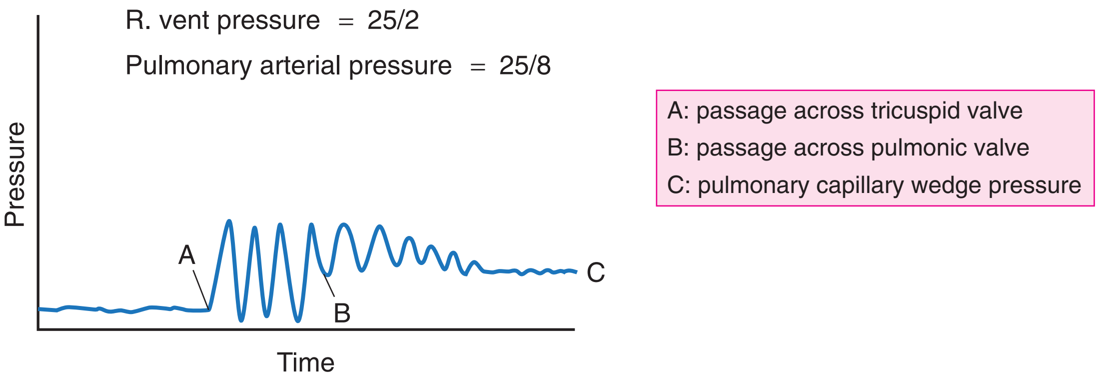


Figure IV-5-5. Swan-Ganz Catheterization

PRESSURE-VOLUME LOOPS

The major features of a left ventricular pressure–volume loop can be seen below. Most of the energy consumption occurs during isovolumetric contraction. Most of the work is performed during the ejection phase.

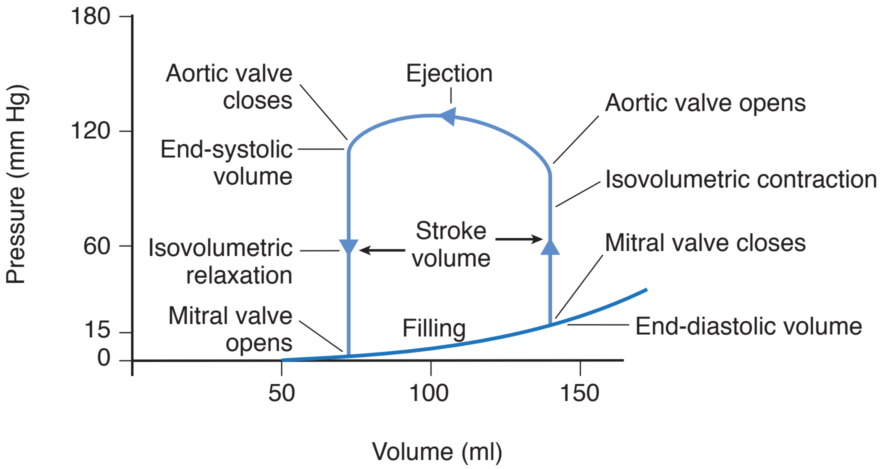


Figure IV-5-6. Left Ventricular Pressure–Volume Loop

Mechanically Altered States

- **Aortic insufficiency**: increased preload, increased stroke volume, increased ventricular systolic pressure (**all cardiac volumes are increased** [EDV, ESV, SV])
- **Heart failure (decreased contractility)**: decreased ventricular systolic pressure, increased preload, loop shifts to the right
- **Essential hypertension (aortic stenosis)**: increased ventricular systolic pressure, little change in preload in the early stages
- **Increased contractility**: increased ventricular systolic pressure, decreased preload, increased ejection fraction, loop shifts to the left
- **Exercise**: increased ventricular systolic pressure, ejection fraction, and preload.

Recall Question

Which of the following is seen in a pressure volume loop in patients with aortic stenosis?

A. Increased preload, increased stroke volume, increased ventricular systolic pressure

B. Decreased ventricular systolic pressure, increased preload, shifts to the right

C. Increased ventricular systolic pressure with little change in preload in the early stages

D. Increased ventricular systolic pressure, decreased preload, increased ejection fraction, loop shifts to the left

E. Increased ventricular systolic pressure, ejection fraction, and preload

Answer: C

VALVULAR DYSFUNCTION

Stenosis of valves usually consists of chronic problems which develop slowly over time. Valvular insufficiency problems can be acute or chronic, the consequences of which can be quite different.

Aortic Stenosis

Aortic stenosis is a pathologic thickening and fusion of the valve leaflets that decrease the open valve area, creating a major resistance point in series with the systemic circuit. There is a large loss in pressure moving the blood through the narrow opening.

- Ventricular systolic pressure increases (increased afterload) to overcome the increased resistance of the aortic valve.
- Pressure overload of the left ventricle leads to a compensatory concentric hypertrophy (new sarcomeres laid down in parallel so that the myofibril thickens) which leads to decreased ventricular compliance (diastolic dysfunction) and coronary perfusion problems and eventually systolic dysfunction.
- Prominent "a" wave of the left atrium as the stiffer left ventricle becomes more dependent on atrial contraction for filling.
- Mean aortic pressure is maintained in the normal range in the early stages of the disorder. Arterial pressure rises slowly and the pulse pressure is reduced.
- There is a pressure gradient between the left ventricle and aorta during ejection.
- Systolic murmur that begins after S1 (midsystolic) which is crescendo-decrescendo in intensity.
- Slow closure of the aortic valve can cause a paradoxical splitting of the second heart sound (aortic valve closes after the pulmonic)

Note

With valvular problems, note the following:

- A **stenotic valve** is a resistor and creates a murmur when the valve is open.
- A **regurgitant valve** allows backflow of blood and creates a murmur when the valve is normally closed.
- Pressure and volume "behind" the defective valve increase. *Behind* refers to the direction of blood flow, e.g., left ventricle is behind the aortic valve; left atrium is behind the mitral valve, etc.

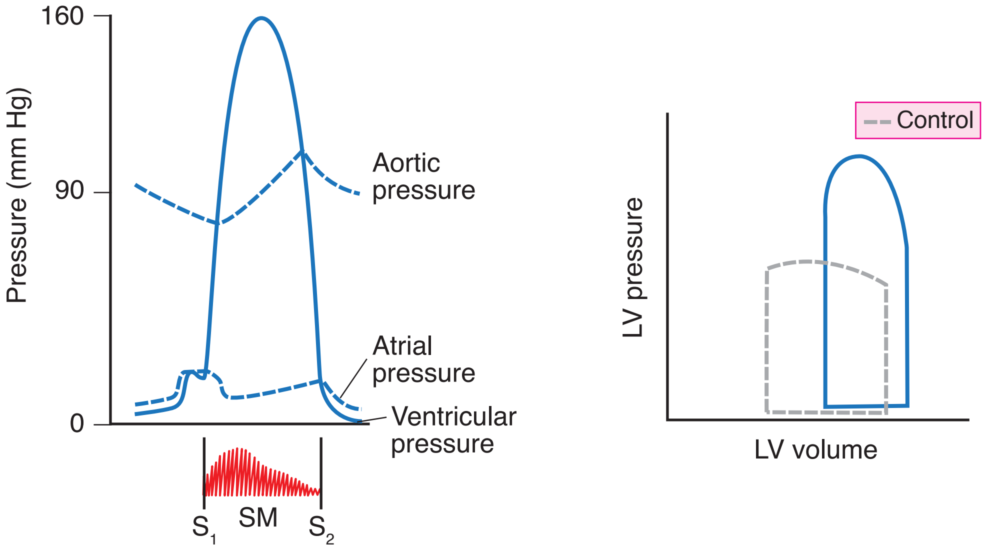


Figure IV-5-7. Aortic Stenosis

Aortic Insufficiency Regurgitation

The aortic valve does not close properly at the beginning of diastole. As a result, during diastole there is retrograde flow from the aorta into the ventricle.

- Acute insufficiency does not allow development of compensatory mechanisms, which can lead to pulmonary edema and circulatory collapse.
- Very large left ventricles are seen in aortic insufficiency. There is a large increase in LVEDV (increase preload) but close to normal end diastolic pressures (eccentric hypertrophy). All cardiac volumes are increased (EDV, ESV, SV).
- Ventricular failure raises pulmonary pressures and causes dyspnea.
- Increased preload causes increased stroke volume, which results in increased ventricular and aortic systolic pressures.
- Retrograde flow from the aorta to the left ventricle produces a low aortic diastolic pressure (the volume of blood left in the aorta at the end of diastole is rapidly reduced).
- There is no true isovolumetric relaxation and a reduced period of isovolumetric contraction.
- Aortic insufficiency is characterized by a large aortic pulse pressure and a low aortic diastolic pressure (hence the bounding pulse).
- Dilation of the ventricle produces a compensatory eccentric hypertrophy.

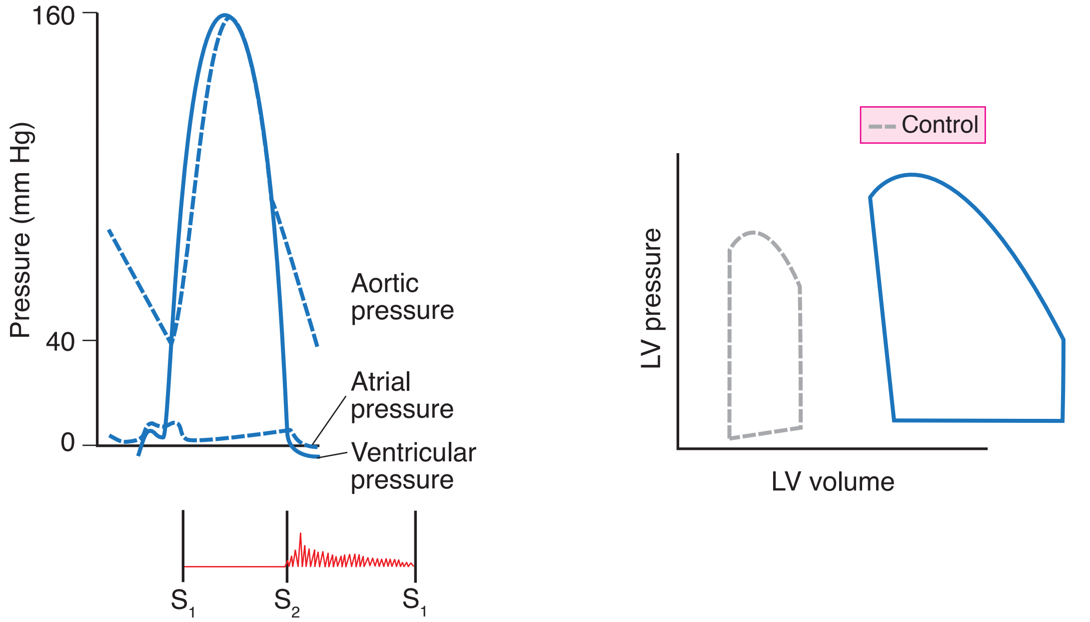


Figure IV-5-8. Aortic Insufficiency (Regurgitation)/(Diastolic Rumble ≈ Austin Flint Murmur)

Mitral Stenosis

A narrow mitral valve impairs emptying of the left atrium (LA) into the left ventricle (LV) during diastole. This creates a pressure gradient between the atrium and ventricle during filling.

- Pressure and volume can be dramatically elevated in the left atrium, dilation of the left atrium over time, which is accelerated with atrial fibrillation.
- Thrombi appear in the enlarged left atrium
- Left atrial pressures are elevated throughout the cardiac cycle. Increased left atrial pressures transmitted to the pulmonary circulation and the right heart.
- Little change or a decrease in the size of the left ventricle. Systolic function normal.
- Diastolic murmur begins after S2 and is associated with altered atrial emptying; a late diastolic murmur and an exaggerated “a” wave are associated with atrial contraction.

Clinical Correlate

The opening snap (OS) to S2 interval is inversely related to left atrial pressure. A **short OS:S2 interval** is a reliable indicator of **severe mitral stenosis**.

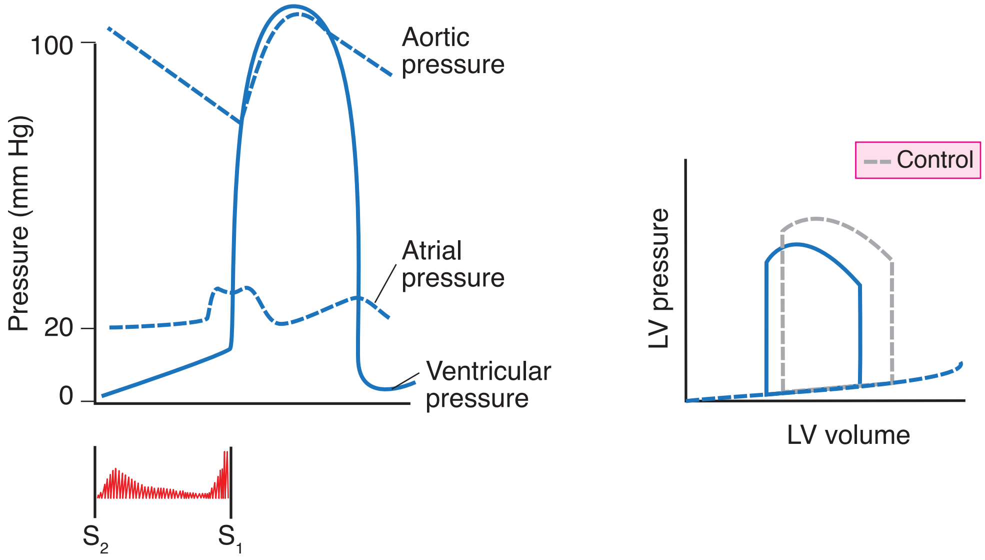


Figure IV-5-9. Mitral Stenosis

Mitral Insufficiency Regurgitation

Acute mitral insufficiency can cause a sudden dramatic rise in pulmonary pressures and pulmonary edema. It can result from structural abnormalities in the valve itself, papillary muscles, chordae tendinae, or a structural change in the mitral annulus.

- No true isovolumetric contraction. Regurgitation of blood from the left ventricle to the left atrium throughout ventricular systole.
- Atrial volumes and pressures increased but chronic dilation of the atrium prevents a dramatic rise in atrial pressures.
- Ventricular volumes and pressures are increased during diastole. Most patients develop chronic compensated left ventricular dilation and hypertrophy, then at some point the left ventricle cannot keep up with the demand and decompensated heart failure develops.
- Increased preload but with reduced afterload.
- Systolic murmur that begins at S_1 (pansystolic).

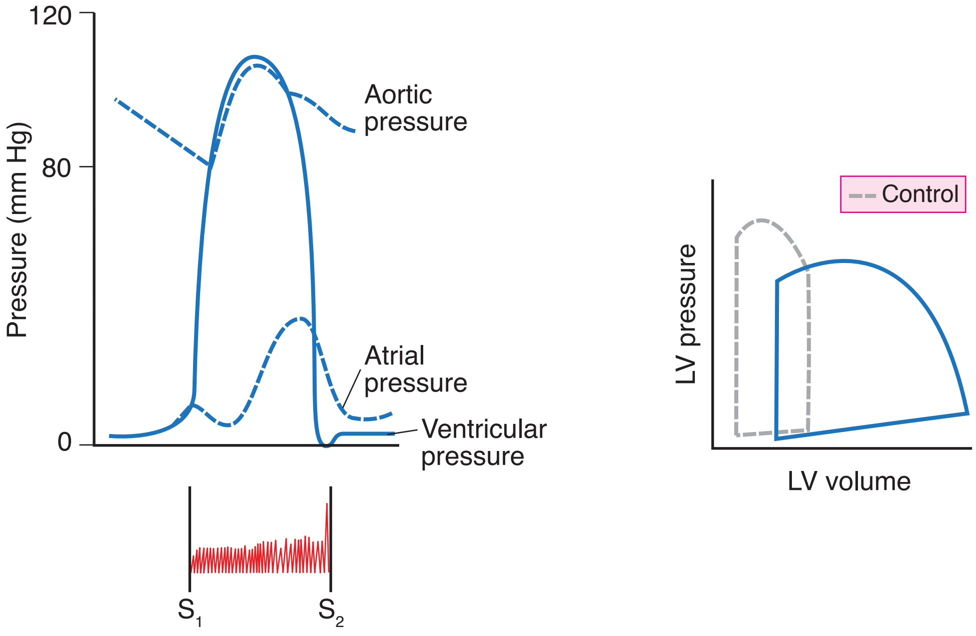


Figure IV-5-10. Mitral Insufficiency (Regurgitation)

Recall Question

Which of the following heart murmurs will be heard in a patient with aortic stenosis?

A. Decrescendo diastolic murmur

B. Low pitched diastolic rumble with an opening snap

C. Holosystolic murmur

D. Crescendo-decrescendo systolic murmur

E. Midsystolic murmur

Answer: D

PART V

Respiration

Lung Mechanics 1

Learning Objectives

- ❑ Answer questions about overview of the respiratory system
- ❑ Interpret scenarios on lung volumes and capacities
- ❑ Solve problems concerning ventilation
- ❑ Use knowledge of lung mechanics
- ❑ Answer questions about cardiovascular changes with ventilation
- ❑ Solve problems concerning positive-pressure ventilation
- ❑ Answer questions about pneumothorax
- ❑ Use knowledge of lung compliance
- ❑ Interpret scenarios on airway resistance
- ❑ Explain information related to pulmonary function testing

THE RESPIRATORY SYSTEM

The purpose of understanding lung mechanics is to view them in the big clinical picture of pulmonary function test (PFT) interpretation. The **PFT is the key diagnostic test for the pulmonologist**, just as the EKG is to the cardiologist.

PFTs consist of 3 individual tests (*see* Respiratory section for more detail):

- Measurements of static lung compartments (i.e., lung volumes)
- Airflow used to evaluate dynamic compliance using a spirometer
- Alveolar membrane permeability using carbon monoxide as a marker of diffusion

LUNG VOLUMES AND CAPACITIES

The figure below graphically shows the relationships among the various lung volumes and capacities. Clinical measurements of specific volumes and capacities provide insights into lung function and the origin of disease processes.

The values for the volumes and capacities given below are typical for a 70 kg male.

Tidal volume (Vt): amount of air that enters or leaves the lung in a single respiratory cycle (500 mL)

Functional residual capacity (FRC): amount of gas in the lungs at the end of a passive expiration; the neutral or equilibrium point for the respiratory system (2,700 mL) ; it is a marker for lung compliance

Inspiratory capacity (IC): maximal volume of gas that can be inspired from FRC (4,000 mL)

Inspiratory reserve volume (IRV): additional amount of air that can be inhaled after a normal inspiration (3,500 mL)

Expiratory reserve volume (ERV): additional volume that can be expired after a passive expiration (1,500 mL)

Residual volume (RV): amount of air in the lung after a maximal expiration (1,200 mL)

Vital capacity (VC): maximal volume that can be expired after a maximal inspiration (5,500 mL)

Total lung capacity (TLC): amount of air in the lung after a maximal inspiration (6,700 mL)

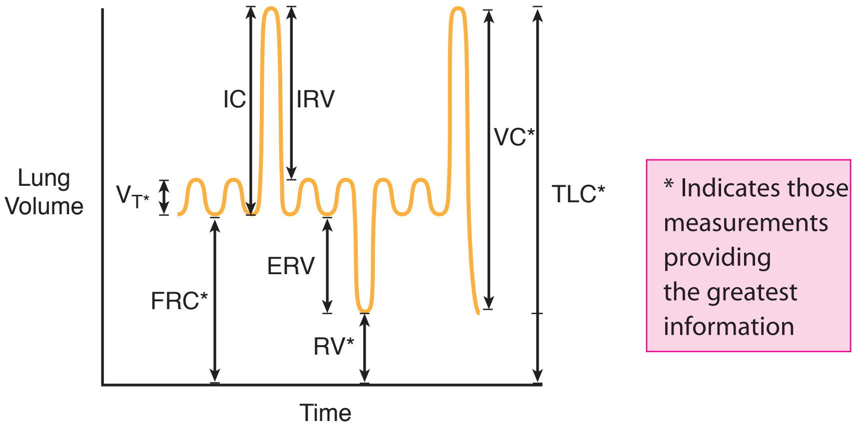


Figure V-1-1. Lung Volumes and Capacities

A spirometer can measure only changes in lung volume. As such, it cannot measure residual volume (RV) or any capacity containing RV. Thus, TLC and FRC cannot be measured using simple spirometry; an indirect method must be used. Common indirect methods are helium dilution, nitrogen washout, and plethysmography.

VENTILATION

Total Ventilation

Total ventilation is also referred to as minute volume or minute ventilation. It is the total volume of air moved in or out (usually the volume expired) of the lungs per minute.

$\dot{V}_E$: total ventilation

V_T: tidal volume

f: respiratory rate

$$\dot{V}_E = V_T \times f$$

Normal resting values would be:

$V_T = 500$ mL

$f = 15$

500 mL $\times$ 15/min $=$ 7,500 mL/min

Note

What is the function of functional residual capacity (FRC)?

Answer: Breathing is cyclic, while blood flow through the pulmonary capillary bed is continuous. During the respiratory cycle, there are short periods of apneas at the end of inspiration and expiration when there is no ventilation but there is continuous blood flow. Without the FRC acting as a buffer for continued gas exchange during apneic periods, these conditions would in effect create an intrapulmonary shunt, inducing deoxygenated blood from the pulmonary capillaries to empty into the pulmonary veins.

Dead Space

Regions of the respiratory system that contain air but are not exchanging O_2 and CO_2 with blood are considered dead space.

Anatomic dead space

Airway regions that, because of inherent structure, are not capable of O_2 and CO_2 exchange with the blood. Anatomic dead space (anatV_D) includes the conducting zone, which ends at the level of the terminal bronchioles. Significant gas exchange (O_2 uptake and CO_2 removal) with the blood occurs only in the alveoli.

The size of the anatV_D in mL is approximately equal to a person's weight in pounds. Thus a 150-lb individual has an anatomic dead space of 150 mL.

Composition of the anatomic dead space and the respiratory zone

The respiratory zone is a very constant environment. Under resting conditions, rhythmic ventilation introduces a small volume into a much larger respiratory zone. Thus, the partial pressure of gases in the alveolar compartment changes very little during normal rhythmic ventilation.

Composition at the End of Expiration (Before Inspiration)

- At the end of an expiration, the anatV_D is filled with air that originated in the alveoli or respiratory zone.
- Thus, the composition of the air in the entire respiratory system is the same at this static point in the respiratory cycle.
- This also means that a sample of expired gas taken near the end of expiration (end tidal air) is representative of the respiratory zone.

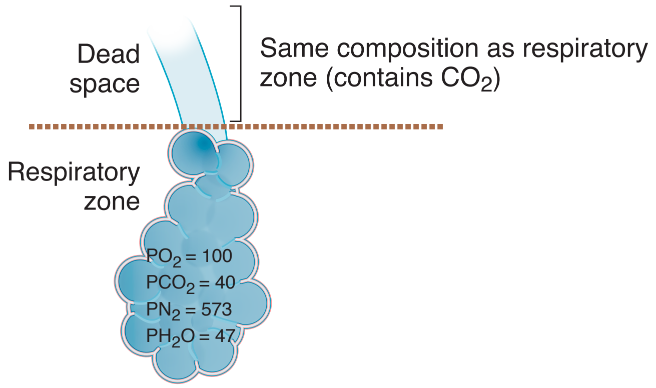


Figure V-1-2. End of Expiration

Composition at the End of Inspiration (Before Expiration)

- The first 150 mL of air to reach the alveoli comes from the anatV_D.
- It is air that remained in the dead space at the end of the previous expiration and has the same composition as alveolar gas.
- After the first 150 mL enters the alveoli, room air is added to the respiratory zone.
- At the end of inspiration the anatV_D is filled with room air.
- The presence of the anatV_D implies the following: in order to get fresh air into the alveoli, one must always take a tidal volume larger than the volume of the anatV_D.

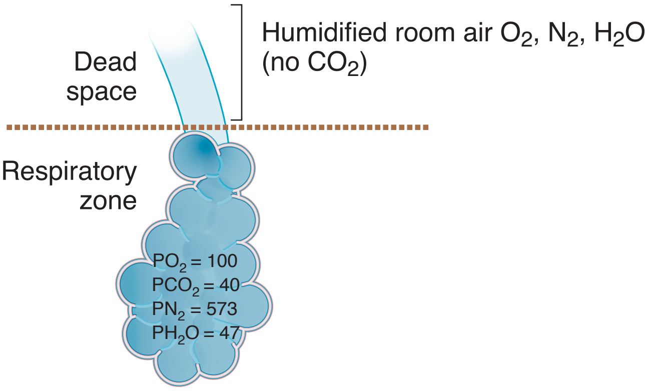


Figure V-1-3. End of Inspiration

Alveolar dead space

Alveolar dead space (alvV_D) refers to alveoli containing air but without blood flow in the surrounding capillaries. An example is a pulmonary embolus.

Physiologic dead space

Physiologic dead space (physioIV_D) refers to the total dead space in the lung system (anatV_D + alvV_D). When the physiol V_D is greater than the anatV_D, it implies the presence of alvV_D, i.e., somewhere in the lung, alveoli are being ventilated but not perfused.

Total ventilation

$$V = VT\ (f)$$
$$= 500\ (15)$$
$$= 7{,}500 \text{ mL/min}$$

Minute ventilation ($\dot{V}$) is the total volume of air entering the lungs per minute.

Alveolar Ventilation

Alveolar ventilation $\dot{V}_A$ represents the room air delivered to the respiratory zone per breath.

- The first 150 mL of each inspiration comes from the anatomic dead space and does not contribute to alveolar ventilation.
- However, every additional mL beyond 150 does contribute to alveolar ventilation.

$$\dot{V}_A = (V_T - V_D)\ f$$
$$= (500 \text{ mL} - 150 \text{ mL})\ 15 = 5250 \text{ mL/min}$$

The alveolar ventilation per inspiration is 350 mL. This equation implies that the volume of fresh air that enters the respiratory zone per minute depends on the pattern of breathing (how large a V_T and the rate of breathing).

$\dot{V}_A$: alveolar ventilation

V_T: tidal volume

V_D: dead space

f: respiratory rate

Increases in the Depth of Breathing

There are equal increases in total and alveolar ventilation per breath, since dead space volume is constant.

If the depth of breathing increases from a depth of 500 mL to a depth of 700 mL, the increase in total and alveolar ventilation is 200 mL per breath.

Increases in the Rate of Breathing

There is a greater increase in total ventilation per minute than in alveolar ventilation per minute, because the increased rate causes increased ventilation of dead space and alveoli.

For every additional inspiration with a tidal volume of 500 mL, total ventilation increases 500 mL, but alveolar ventilation only increases by 350 mL (assuming dead space is 150 mL).

For example, given the following, which person has the greater alveolar ventilation?

	Tidal Volume	Rate	Total Ventilation
Person A	600 mL	10/min	6,000 mL/min
Person B	300 mL	20/min	6,000 mL/min

Answer: Person A. Person B has rapid, shallow breathing. This person has a large component of dead-space ventilation (first 150 mL of each inspiration). Even though total ventilation may be normal, alveolar ventilation is decreased. Therefore, the individual is hypoventilating.

In rapid, shallow breathing, total ventilation may be above normal, but alveolar ventilation may be below normal.

LUNG MECHANICS

Muscles of Respiration

Inspiration

The major muscle of inspiration is the **diaphragm**. Contraction of the diaphragm enlarges the vertical dimensions of the chest. Also utilized are the external intercostal muscles of the chest wall. Contraction of these muscles causes the ribs to rise and thus increases the anterior-posterior dimensions of the chest.

Expiration

Under resting conditions, expiration is normally a passive process, i.e., it is due to the relaxation of the muscles of inspiration and the elastic recoil of the lungs. For a forced expiration, the muscles of the abdominal wall and the internal intercostals contract. This compresses the chest wall down and forces the diaphragm up into the chest.

Included would be external oblique, rectus abdominal, internal oblique, and transverse abdominal muscles.

Forces Acting on the Lung System

In respiratory physiology, **units of pressure** are usually given as cm H_2O.

$$1 \text{ cm } H_2O = 0.74 \text{ mm Hg } (1 \text{ mm Hg} = 1.36 \text{ cm } H_2O)$$

Lung recoil and intrapleural pressure

Understanding lung mechanics involves understanding the main forces acting on the respiratory system.

Lung recoil represents the inward force created by the elastic recoil properties of alveoli.

- As the lung expands, recoil increases; as the lung gets smaller, recoil decreases.
- Recoil, as a force, always acts to collapse the lung.

Chest wall recoil represents the outward force of the chest wall.

- FRC represents the point where this outward recoil of the chest wall is counterbalanced by the inward recoil of the lung.

Intrapleural pressure (IPP) represents the pressure inside the thin film of fluid between the visceral pleura, which is attached to the lung, and the parietal pleura, which is attached to the chest wall.

- The outward recoil of the chest and inward recoil of the lung create a negative (subatmospheric) IPP.
- IPP is the outside pressure for all structures inside the chest wall.

Transmural pressure gradient (P_{TM}) represents the pressure gradient across any tube or sphere.

- Calculated as inside pressure minus outside pressure
- If positive (inside greater than outside), it is a net force pushing out against the walls of the structure
- If negative (outside greater than inside), it is a net force pushing in against the walls of the structure; depending upon the structural components, the tube/sphere can collapse if P_{TM} is negative or zero
- At FRC, IPP is negative, and thus P_{TM} is positive. This positive outward force prevents alveolar collapse (atelectasis).
- For the entire lung, P_{TM} is called the transpulmonary pressure (TPP).

Before Inspiration

The glottis is open, and all respiratory muscles are relaxed (FRC). This is the neutral or equilibrium point of the respiratory system. Intrapleural pressure is negative at FRC because the inward elastic recoil of the lungs is opposed by the outward-directed recoil of the chest wall.

Because no air is flowing through the open glottis, alveolar pressure must be zero. By convention, the atmospheric pressure is set to equal zero.

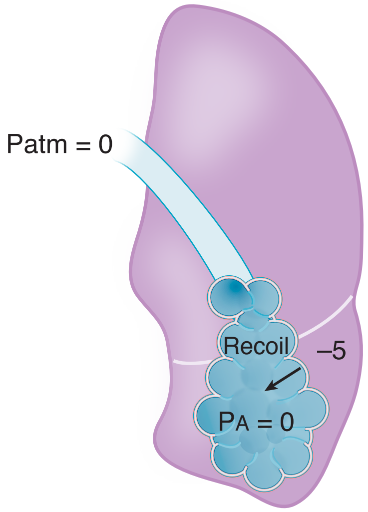


Figure V-1-4. Lung Force Relationships at FRC

Intrapleural pressure: –5 cm H_2O

PTM: 5

Alveolar pressure: 0

During Inspiration

Inspiration is induced by the contraction of the diaphragm and external intercostal muscles that expand the chest wall. The net result is to make intrapleural pressure more negative.

- The more negative IPP causes P_{TM} (TPP) to increase, which in turn causes expansion of the lungs. The greater the contraction, the greater the change in intrapleural pressure and the larger the P_{TM} (TPP) expanding the lung.
- The expansion of the lung increases alveolar volume. Based upon Boyle's law, the rise in volume causes pressure to decrease, resulting in a negative (subatmospheric) alveolar pressure.
- Because alveolar pressure is now less than atmospheric, air rushes into the lungs.

End of Inspiration

The lung expands until alveolar pressure equilibrates with atmospheric pressure. The lungs are at their new, larger volume. Under resting conditions, about 500 mL of air flows into the lung system in order to return alveolar pressure back to zero.

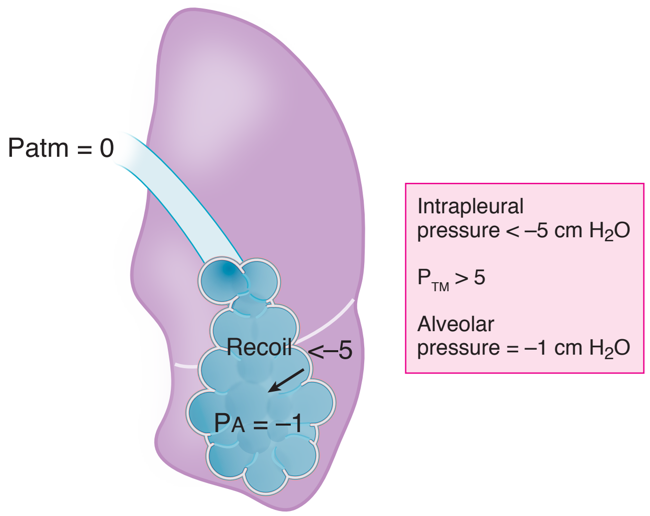


Figure V-1-5. Lung Forces during Inspiration

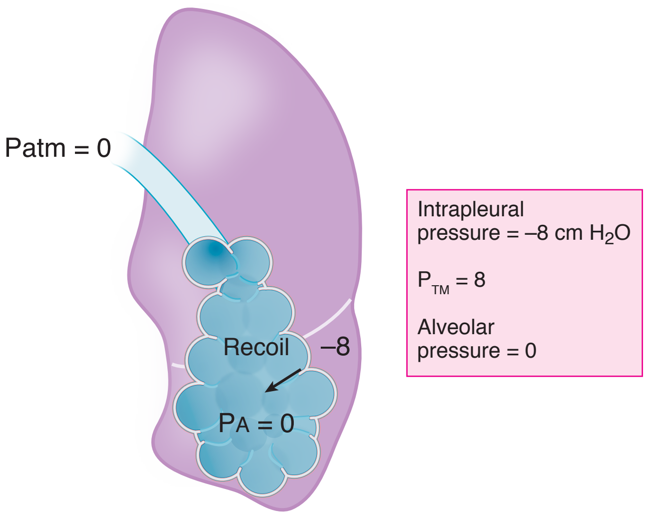
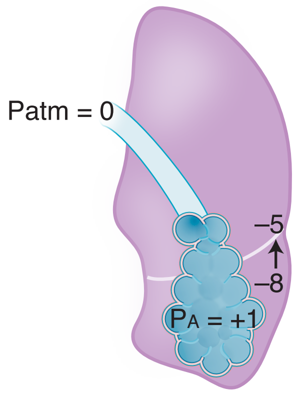


Figure V-1-6. Lung Forces at End of Inspiration



Expiration

Expiration under resting conditions is produced simply by the relaxation of the muscles of inspiration.

- Relaxation of the muscles of inspiration causes intrapleural pressure to return to –5 cm H_2O
- This decreases IPP back to its original level of –5 cm H_2O, resulting in a decreased P_{TM}. The drop in P_{TM} reduces alveolar volume, which increases alveolar pressure (Boyle's law).
- The elevated alveolar pressure causes air to flow out of the lungs. The outflowing air returns alveolar pressure toward zero, and when it reaches zero, airflow stops. The lung system returns to FRC.

The **intrapleural pressure** during a normal respiratory cycle is illustrated below. Under resting conditions, it is always a subatmosphere pressure.

The **intraalveolar pressure** during a normal respiratory cycle is also illustrated below. It is slightly negative during inspiration and slightly positive during expiration.

- No matter how large a breath is taken, intraalveolar pressure always returns to 0 at the end of inspiration and expiration.
- By convention, total atmospheric pressure = 0.

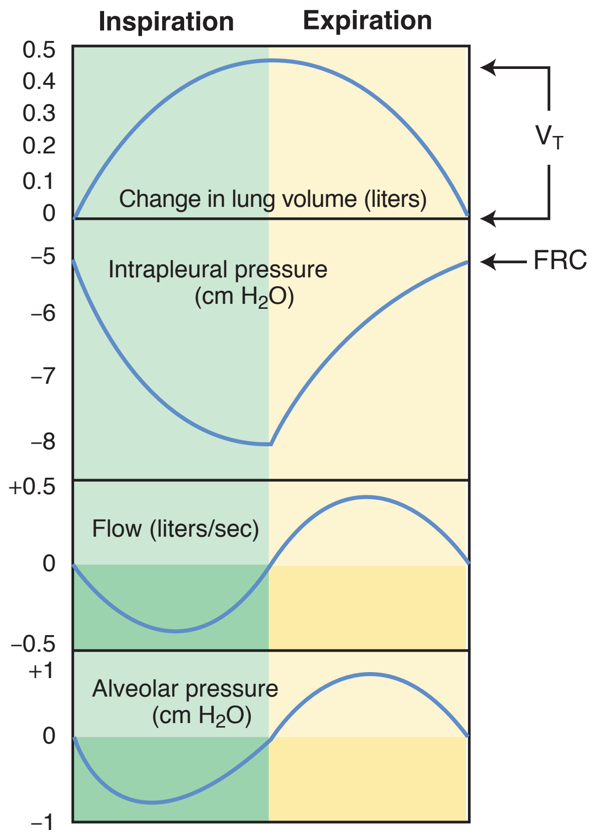


Figure V-1-7. Essentials of Pulmonary Events during a Breath

Recall Question

The following lung volumes are noted on spirometer of a 38-year-old man with asthma: FRC 3.0 L, VC 6.0 L, and ERV 1.5 L. What is this patient's IC?

A. 9.0 L

B. 7.5 L

C. 4.5 L

D. 3.0 L

E. Value cannot be determined

Answer: C

CARDIOVASCULAR CHANGES WITH VENTILATION

Inspiration

With inspiration, **intrapleural pressure becomes more negative (decreases).** This increases the P_{TM} across the vasculature, causing the great veins and right atrium to expand. This expansion decreases intravascular pressure, thereby increasing the pressure gradient driving VR to the right heart.

- Systemic venous return and right ventricular output are increased.
- An increase in the output of the right ventricle delays closing of the pulmonic valves and typically results in a splitting of the second heart sound.
- Pulmonary vessels expand, and the volume of blood in the pulmonary circuit increases. In addition, because pulmonary vascular resistance (PVR) is lowest at FRC, it increases.
- In turn, venous return to the left heart, and the output of the left ventricle is decreased, causing decreased systemic arterial pressure (drop in systolic most prominent).
- This inspiration reduces vagal outflow to the heart (mechanism debatable) resulting in a slight rise in heart rate (respiratory sinus arrhythmia). This is why patients are asked to hold their breath, if clinically possible, when an EKG is taken.

Expiration

Expiration is the reverse of the processes above. **Intrapleural pressure becomes more positive (increases)**, i.e., returns to original negative value. P_{TM} returns to its original level, thereby decreasing the pressure gradient for VR.

- Systemic venous return and output of the right ventricle are decreased.
- Pulmonary vessels are compressed, and the volume of blood in the pulmonary circuit decreases.
- The return of blood and output of the left ventricle increases, causing systemic arterial pressure to rise (primarily systolic).
- Vagal outflow increases (mechanism debated), reducing HR (respiratory sinus arrhythmia).
- A Valsalva maneuver is a forced expiration against a closed glottis. This forced expiration creates a positive IPP (see later in this chapter), which compresses the great veins in the chest. This in turn reduces VR.

POSITIVE-PRESSURE VENTILATION

Assisted Control Mode Ventilation (ACMV)

In ACMV, the inspiratory cycle is initiated by patient or automatically if no signal is detected within a specified time window. Expiration is not assisted. Expiration is accomplished in the normal manner (passive recoil of the lungs).

Positive End-Expiratory Pressure (PEEP)

In PEEP, positive pressure is applied at the end of the expiratory cycle to decrease alveolar collapse. It is useful in treating the hypoxemia of acute respiratory distress syndrome (ARDS) (*see* Hypoxemia section.)

- Small alveoli have a strong tendency to collapse, creating regions of atelectasis.
- The larger alveoli are also better ventilated, and supplementary oxygen is more effective at maintaining a normal arterial PO_2.
- One downside to positive pressure ventilation and accentuated by PEEP is a decrease in venous return and cardiac output.

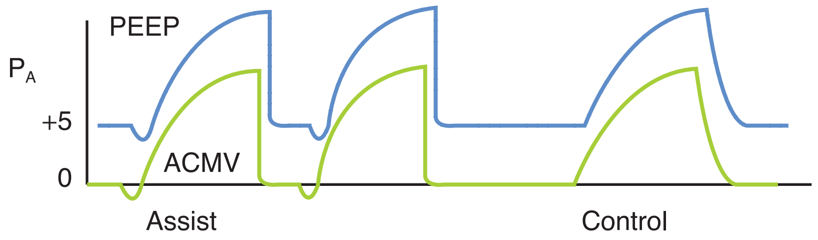


Figure V-1-8a. Positive-Pressure Ventilation

Continuous Positive Airway Pressure (CPAP)

In CPAP, continuous positive pressure is applied to the airways. It is useful in treating obstructive sleep apnea (OSA) since the lung and upper airways (nasopharynx) remain at a larger volume throughout the respiratory cycle.

CPAP is administered by mask (patient not intubated). The patient breathes spontaneously.

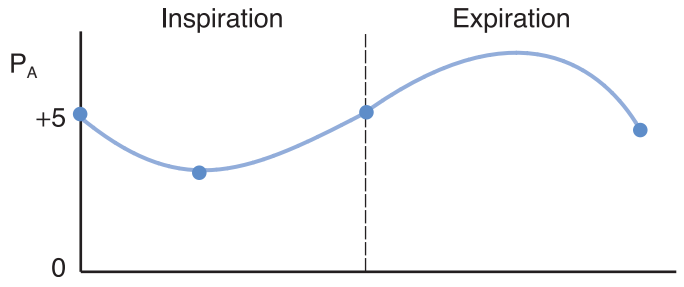


Figure V-1-8b. CPAP

PNEUMOTHORAX

The following changes occur with the development of a simple pneumothorax. The pneumothorax may be **traumatic** (perforation of chest wall) or **spontaneous** (rupture of an alveolus):

- Intrapleural pressure increases from a mean at –5 cm H_2O to equal atmospheric pressure.
- Lung recoil decreases to zero as the lung collapses.
- Chest wall expands. At FRC, the chest wall is under a slight tension directed outward. It is this tendency for the chest wall to spring out and the opposed force of recoil that creates the intrapleural pressure of –5 cm H_2O.
- Transpulmonary pressure is negative.

In some cases, the opening of the lung to the pleural space may function as a valve allowing the air to enter the pleural space but not to leave. This creates a tension pneumothorax.

- Strong inspiratory efforts promote the entry of air into the pleural space, but during expiration, the valve closes and positive pressures are created in the chest cavity. Ventilation decreases but the positive pressures also decrease venous return and cardiac output.
- Tension pneumothorax most commonly develops in patients on a positive-pressure ventilator.

Clinical Correlate

Common clinical signs of a tension pneumothorax include:

- Respiratory distress
- Asymmetry of breath sounds
- Deviation of trachea to the side opposite the tension pneumothorax
- Markedly depressed cardiac output

LUNG COMPLIANCE

A static isolated lung inflation curve is illustrated below.

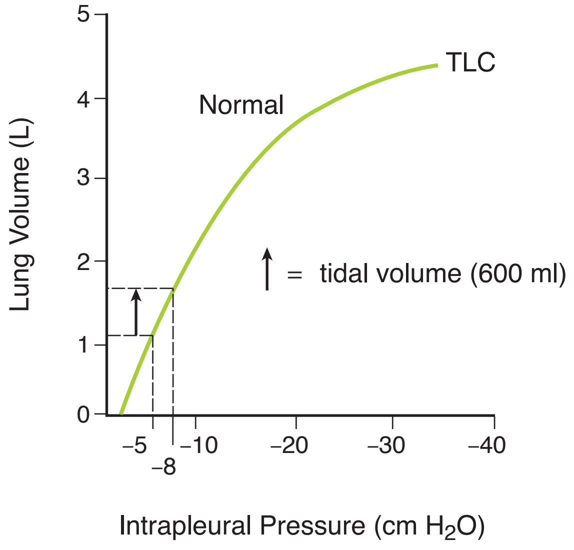


Figure V-1-9. Lung Inflation Curve

Lung compliance is the change in lung volume (tidal volume) divided by the change in surrounding pressure. This is stated in the following formula:

$$\textbf{Compliance} = \frac{\Delta V}{\Delta P}$$

Problem

Tidal volume = 0.6 liters

Intrapleural pressure before inspiration = −5 cm H_2O

Intrapleural pressure after inspiration = −8 cm H_2O

$$\text{Lung compliance} = \frac{0.6 \text{ liters}}{3 \text{ cm } H_2O} = 0.200 \text{ liters/cm } H_2O$$

The preceding calculation simply means that for every 1 cm H_2O surrounding pressure changes, 200 mL of air flows in or out of the respiratory system. It flows into the system if surrounding pressure becomes more negative (e.g., –5 to –6 cm H_2O) or out of the system if surrounding pressure becomes more positive (e.g., –5 to –4 cm H_2O).

- Increased compliance means more air will flow for a given change in pressure.
- Reduced compliance means less air will flow for a given change in pressure.
- In the preceding curve, although the slope is changing during inflation, its value at any point is the lung's compliance. It is the relationship between the change in lung volume (tidal volume) and the change in intrapleural or surrounding pressure.
- The steeper the line, the more compliant the lungs. Restful breathing works on the steepest, most compliant part of the curve.
- With a deep inspiration, the lung moves toward the flatter part of the curve, and thus it has reduced compliance. Lung compliance is less at TLC compared to FRC.

The figure below shows pathologic states in which lung compliance changes.

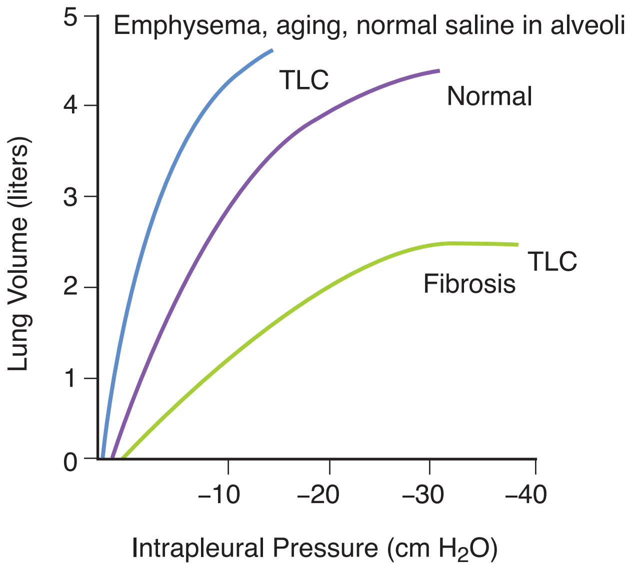


Figure V-1-10. Lung Compliance

Increased lung compliance also occurs with aging and with a saline-filled lung.

- Compliance is an index of the effort required to expand the lungs (to overcome recoil). It does not relate to airway resistance.
- Compliance decreases as the lungs are inflated because the curve is not a straight line.
- For any given fall in intrapleural pressure, large alveoli expand less than small alveoli.
- Very compliant lungs (easy to inflate) have low recoil. Stiff lungs (difficult to inflate) have a large recoil force.

Components of Lung Recoil

Lung recoil has the following components:

- **The tissue itself;** more specifically, the collagen and elastin fibers of the lung
  - The larger the lung, the greater the stretch of the tissue and the greater the recoil force.
- **The surface tension forces in the fluid lining the alveoli.** Surface tension forces are created whenever there is a liquid–air interface.
  - Surface tension forces tend to reduce the area of the surface and generate a pressure. In the alveoli, they act to collapse the alveoli; therefore, these forces contribute to lung recoil.
- **Surface tension forces are the greatest component of lung recoil.** The relationship between the surface tension and the pressure inside a bubble is given by the Law of LaPlace.

$$\text{Pressure} \propto \frac{\text{tension}}{\text{radius}}$$

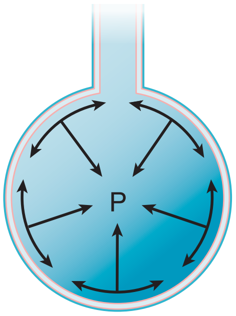


Figure V-1-11. Surface Tension

If wall tension is the same in 2 bubbles, the smaller bubble will have the greater pressure.

Although the situation is more complex in the lung, it follows that small alveoli tend to be unstable. They have a great tendency to empty into larger alveoli and collapse (creating regions of atelectasis). Collapsed alveoli are difficult to reinflate.

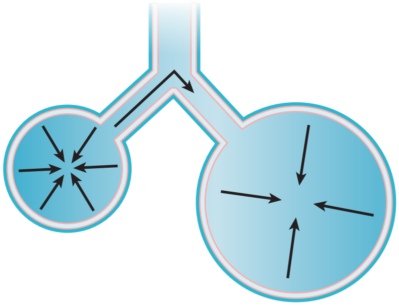


Figure V-1-12. Atelectasis

If the alveoli were lined with a simple electrolyte solution, lung recoil would be so great that lungs theoretically should not be able to inflate. This is prevented by a chemical (produced by alveolar type II cells), surfactant, in the fluid lining a normal lung.

Surfactant has 2 main functions:

- It lowers surface tension forces in the alveoli; in other words, it lowers lung recoil and increases compliance.
- It lowers surface tension forces more in small alveoli than in large alveoli. This promotes stability among alveoli of different sizes by decreasing the tendency of small alveoli to collapse (decreases the tendency to develop atelectasis).

Respiratory Distress Syndrome (RDS)

Infant RDS (hyaline membrane disease) is a deficiency of surfactant.

Adult respiratory distress syndrome (ARDS) is an acute lung injury via the following:

- Bloodstream (sepsis): develops from injury to the pulmonary capillary endothelium, leading to interstitial edema and increased lymph flow
  - Leads to injury and increased permeability of the alveolar epithelium and alveolar edema
  - The protein seepage into the alveoli reduces the effectiveness of surfactant.
  - Neutrophils have been implicated in the progressive lung injury from sepsis.
- Airway (gastric aspirations): direct acute injury to the lung epithelium increases permeability of the epithelium followed by edema

In the figure below, curve A represents respiratory distress syndrome. The curve is shifted to the right, and it is a flatter curve (lung stiffer).

- A greater change in intrapleural pressure is required to inflate the lungs.
- The tendency for collapse is increased, thus PEEP is sometimes provided.

Curve B represents atelectasis.

- Once alveoli collapse, it is difficult to reinflate them.
- Note the high TPP required to open atelectic alveoli (green line, B, in figure below).

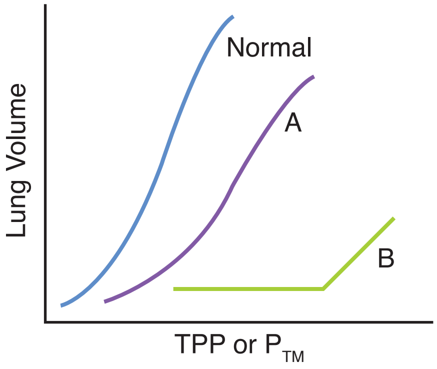


Figure V-1-13. Deficiency of Surfactant

AIRWAY RESISTANCE

Radius of an Airway

In the branching airway system of the lungs, it is the first and second bronchi that represent most of the airway resistance.

- Parasympathetic nerve stimulation produces bronchoconstriction.
- This is mediated by M3 receptors. In addition, M3 activation increases airway secretions.
- Circulating catecholamines produce bronchodilation. Epinephrine is the endogenous agent and it bronchodilates via b2 receptors.

$$\textbf{Resistance} = \frac{\mathbf{1}}{\textbf{radius}^{4}}$$

Mechanical Effect of Lung Volume

The figure below illustrates that, as lung volume increases, airway resistance decreases.

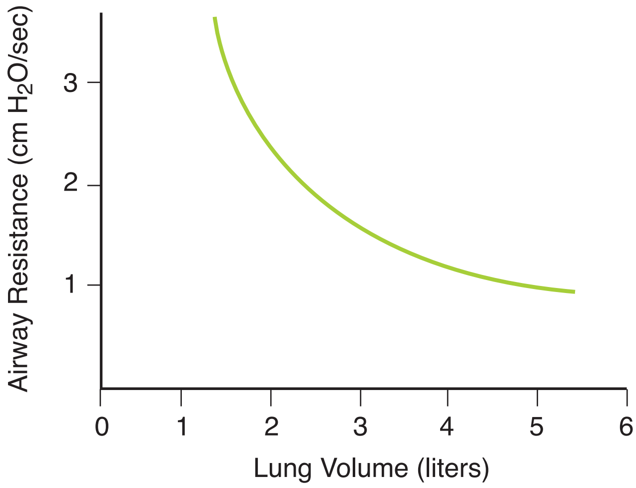


Figure V-1-14. Airway Resistance

The mechanisms for this are:

- **P_{TM}:** To get to high lung volumes, IPP becomes more and more negative. This increases the P_{TM} across small airways, causing them to expand. The result is decreased resistance.
- **Radial traction:** The walls of alveoli are physically connected to small airways. Thus, as alveoli expand, they pull open small airways. The result is decreased resistance.

PULMONARY FUNCTION TESTING

Vital Capacity

Vital capacity (VC) is the maximum volume of air that an individual can move in a single breath. The most useful assessment of the VC is to expire as quickly and forcefully as possible, i.e., a "timed" or forced VC (or FVC). During the FVC maneuver, the volume of air exhaled in the first second is called the forced expiratory volume in 1 sec (FEV_1).

This figure and those that follow differ from the output of a spirometer because they show *actual* lung volume (including residual volume), not only *changes* in volume.

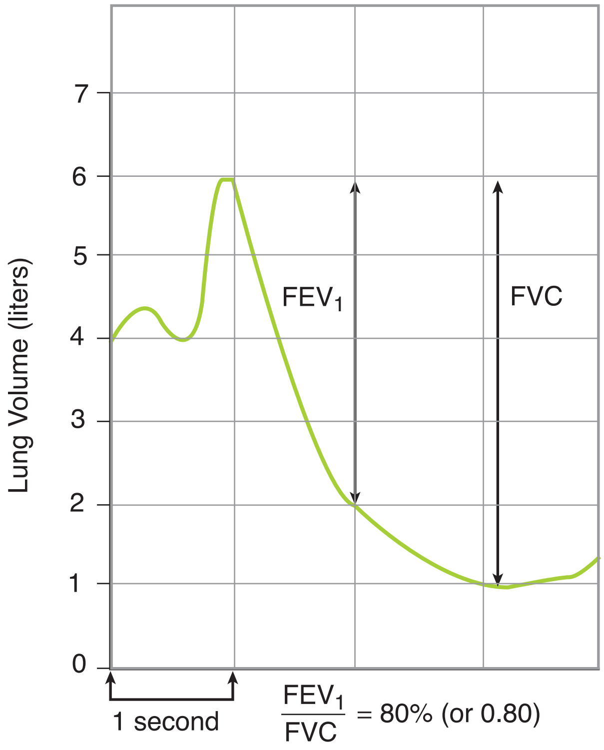


Figure V-1-15. Pulmonary Function Test of Forced Vital Capacity

There are 2 key pieces of data from a PFT involving the measurement of FVC:

- **FVC:** this is total volume exhaled
  - Because age, gender, body size, etc., can influence the absolute amount of FVC, it is expressed as a percent of predicted (100% of predicted being the "ideal").
- **FEV1 (forced expiratory volume in 1 second):** although this volume can provide information on its own, it is commonly compared to the FVC such that one determines the FEV1/FVC ratio.
  - This ratio creates a flow parameter; 0.8 (80%) or greater is considered normal.
- Thus, this PFT provides a volume and a flow.
- **Restrictive** pulmonary disease is characterized by reduced volume (low FVC, but normal flow), while **obstructive** disease is characterized by reduced flow (low FEV1/FVC).

Physiology of a PFT

In the figure below, the picture on the left shows that at the end of an inspiratory effort to TLC, IPP is very negative. This negative IPP exists throughout the lungs during a passive expiration and thus the P_{TM} is positive for both alveoli and airways.

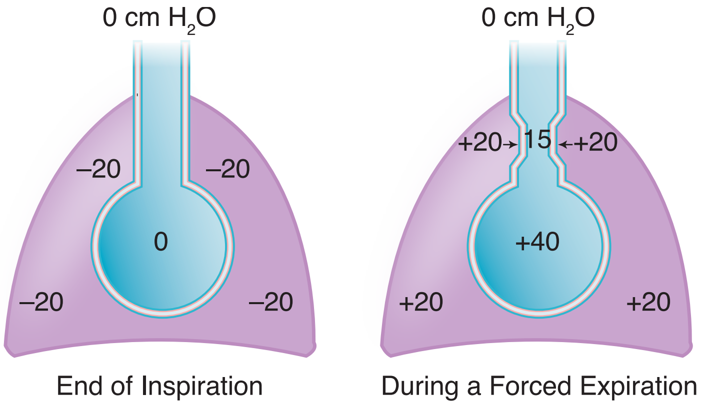


Figure V-1-16. Dynamic Airway Compression

The picture on the right shows the situation during a maximal forced expiration.

- A forced expiration compresses the chest wall down and in, creating a positive IPP. The level of positive IPP generated is dependent upon effort.
- This forced expiration creates a very positive alveolar pressure, in turn creating a large pressure gradient to force air out of the lungs.
- However, this positive IPP creates a negative P_{TM} in the airways. It is more negative in the large airways, e.g., trachea and main-stem bronchi. These regions have structural support and thus do not collapse even though P_{TM} is very negative.
- Moving down the airways toward alveoli, the negative P_{TM} ultimately compresses airways that lack sufficient structural support. This is dynamic compression of airways.
- This compression of airways creates a tremendous resistance to airflow. In fact, the airway may collapse, producing infinite resistance. Regardless, this compression creates a level of resistance that overwhelms any and all other resistors that exist in the circuit and is thus the dominant resistor for airflow.
- Once this occurs, elastic recoil of the lung becomes the effective driving force for airflow and airflow becomes independent of the effort. This means airflow is a property of the patient's respiratory system, hence the reason this test is very diagnostic.
- Because this resistance is created in small airways, the entire volume of the lungs cannot be expired, creating residual volume (RV).

Because PFTs measure flow (FEV1/FVC) and volume, they accurately diagnose obstructive (low flow) and restrictive disease (low volume, normal flow).

Obstructive versus Restrictive Patterns

The following figures demonstrate a standard PFT, the measurement of FVC, FEV_1, and FEV_1/FVC.

Bridge to Pathology

There are 4 basic pathologic alterations that can occur in obstructive disease:

- Bronchoconstriction
- Hypersecretion
- Inflammation
- Destruction of lung parenchyma (emphysema)

Obstructive pulmonary disease

Obstructive disease is characterized by an increase in airway resistance that is measured as a decrease in expiratory flow. Examples are chronic bronchitis, asthma, and emphysema.

Obstructive pattern

- Total lung capacity (TLC) is normal or larger than normal, but during a maximal forced expiration from TLC, a smaller than normal volume is slowly expired.
- Depending upon the severity of the disease, FVC may or may not be reduced. If severe enough, then FVC is diminished.

Bridge to Pharmacology

Treatment of obstructive disease includes β2-agonists (short- and long-acting), M3 blockers such as ipratropium, PDE inhibitors, mast cell stabilizers, leukotriene-receptor blockers, and steroids.

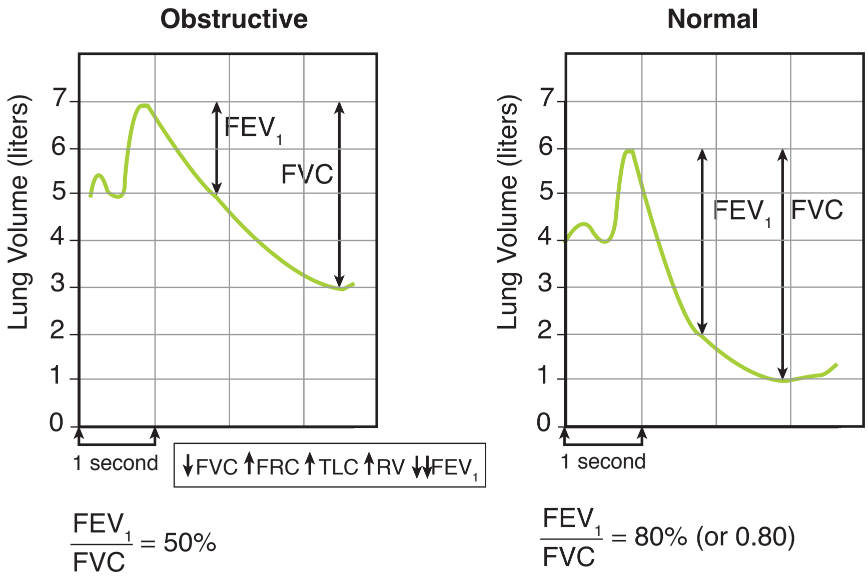


Figure V-1-17. Obstructive Pattern

Restrictive pulmonary disease

Restrictive pulmonary disease is characterized by an increase in elastic recoil—a decrease in lung compliance—which is measured as a decrease in all lung volumes. Reduced vital capacity with low lung volumes are the indicators of restrictive pulmonary diseases. Examples are ARDS and interstitial lung diseases such as sarcoidosis and idiopathic pulmonary fibrosis (IPF).

Restrictive pattern

- TLC is smaller than normal, but during a maximal forced expiration from TLC, the smaller volume is expired quickly and more completely than in a normal pattern.
- Therefore, even though FEV_1 is also reduced, the FEV_1/FVC is often increased.
- However, the critical distinction is low FVC with low FRC and RV.

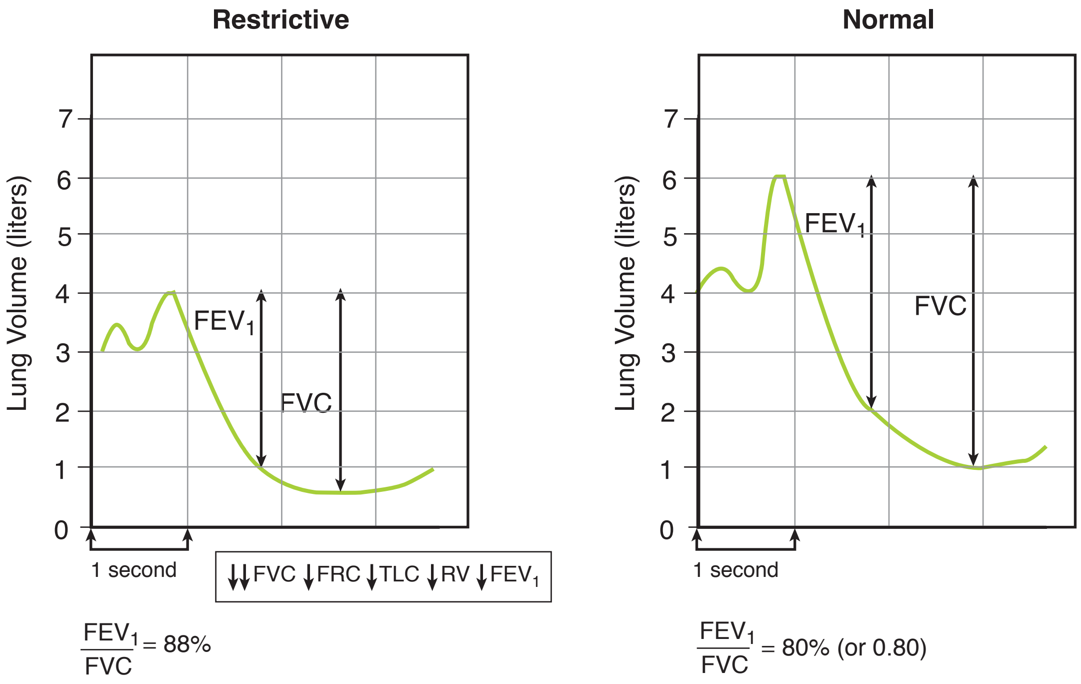


Figure V-1-18. Restrictive Pattern

Table V-1-1. Obstructive Versus Restrictive Pattern

Variable	Obstructive Pattern (e.g., Emphysema)	Restrictive Pattern (e.g., Fibrosis)
TLC	↑	↓↓
FEV_1	↓↓	↓
FVC	↓	↓↓
FEV_1/FVC	↓	↑ or normal
Peak flow	↓	↓
FRC	↑	↓
RV	↑	↓

FVC is always decreased when pulmonary function is significantly compromised.

A decrease in FEV_1/FVC ratio is evidence of an obstructive pattern. A normal or increased FEV_1/FVC ratio is evidence of a restrictive pattern, but a low TLC is diagnostic of restrictive lung disease.

Flow–Volume Loops

The instantaneous relationship between flow (liters/sec) and lung volume is useful in determining whether obstructive or restrictive lung disease is present. In the loop shown below, expiration starts at total lung capacity and continues to residual volume. The width of the loop is the FVC.

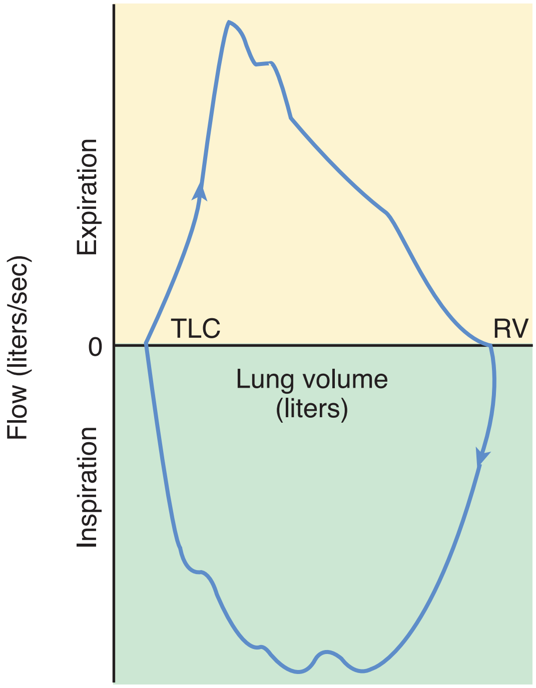


Figure V-1-19. Flow–Volume Loop

Loops found in obstructive and restrictive disease are shown below.

In **obstructive disease**, the flow–volume loop begins and ends at abnormally high lung volumes, and the expiratory flow is lower than normal. In addition, the downslope of expiration "scallops" or "bows" inward. This scalloping indicates that at any given lung volume, flow is less. Thus, airway resistance is elevated (obstructive).

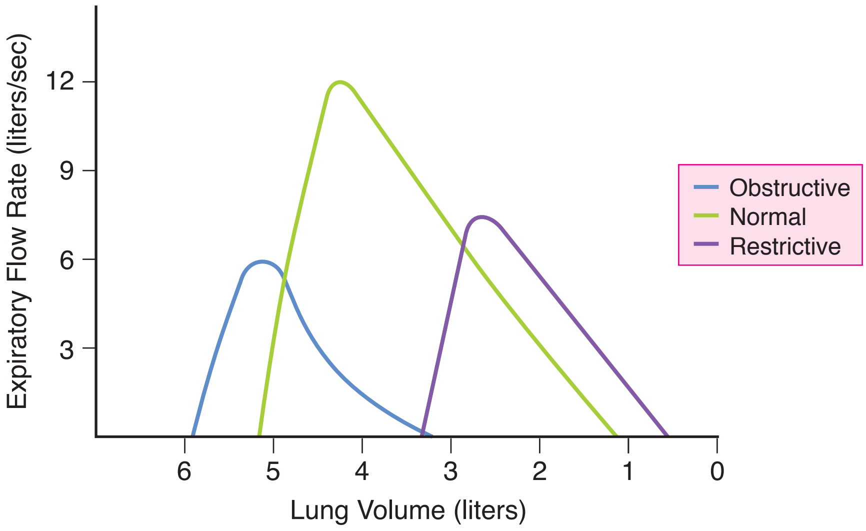


Figure V-1-20. Forced Expiratory Flow–Volume Loop

In **restrictive disease**, the flow–volume loop begins and ends at unusually low lung volumes. Peak flow is less, because overall volume is less. However, when expiratory flow is compared at specific lung volumes, the flow in restrictive disease is somewhat greater than normal.

Recall Question

Which of the following lung diseases decreases total lung capacity on a pulmonary function test?

A. Emphysema

B. Chronic bronchitis

C. Interstitial pulmonary fibrosis

D. Aging

E. Normal saline in alveoli

Answer: C

Alveolar–Blood Gas Exchange 2

Learning Objectives

- ❑ Answer questions about the normal lung
- ❑ Solve problems concerning factors affecting alveolar PCO_2
- ❑ Use knowledge of factors affecting alveolar PO_2
- ❑ Interpret scenarios on alveolar-blood gas transfer: Fick law of diffusion
- ❑ Use knowledge of diffusing capacity of the lung

THE NORMAL LUNG

Partial Pressure of a Gas in Ambient Air

$$Pgas = Fgas \times Patm$$

By convention, the partial pressure of the gas is expressed in terms of its dry gas concentration. For example, the PO_2 in ambient air is:

$$PO_2 = 0.21 \times 760 = 160 \text{ mm Hg}$$

Patm: atmospheric pressure

Pgas: partial pressure of a gas

Fgas: concentration of a gas

Partial Pressure of a Gas in Inspired Air

Inspired air is defined as air that has been inhaled, warmed to 37°C, and completely humidified, but has not yet engaged in gas exchange. It is the fresh air in the anatV_D that is about to enter the respiratory zone.

The partial pressure of H_2O is dependent only on temperature and at 37°C is 47 mm Hg. Humidifying the air reduces the partial pressure of the other gases present.

$$PIgas = Fgas\ (Patm - PH_2O)$$

For example, the PO_2 of inspired air is:

$$PIO_2 = 0.21\ (760 - 47) = 150 \text{ mm Hg}$$

The figure below shows the pressures of oxygen and carbon dioxide in the alveolar, pulmonary end capillary, and systemic arterial blood.

PIgas: partial pressure of inspired gas

PH_2O: partial pressure of H_2O vapor

Note

Dalton's law of partial pressures states that the total pressure exerted by a mixture of gases is the sum of the pressures exerted independently by each gas in the mixture.

Also, the pressure exerted by each gas (its partial pressure) is directly proportional to its percentage in the total gas mixture.

A = alveolar
a = systemic arterial

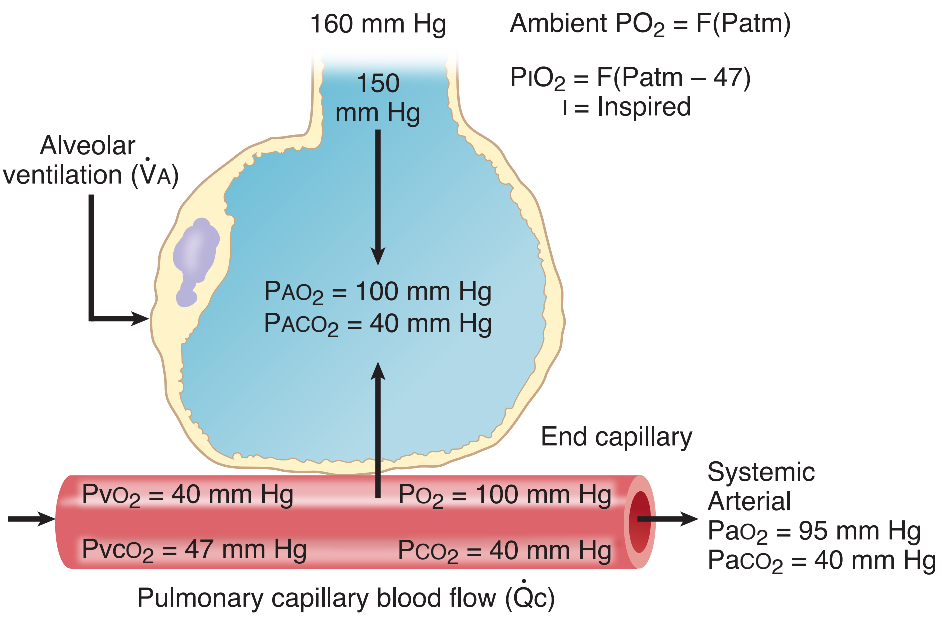


Figure V-2-1. Pulmonary Capillary Gases

- Under normal conditions, the PO_2 and PCO_2 in the alveolar compartment and pulmonary end capillary blood are the same (perfusion-limited).
- There is a slight change ($PO_2\downarrow$) between the end capillary compartment and systemic arterial blood because of a small but normal shunt through the lungs.
- Alveolar–systemic arterial PO_2 differences = A – a O_2 gradient.
- This difference (5–10 mm Hg) often provides information about the cause of a hypoxemia.

FACTORS AFFECTING ALVEOLAR PCO_2

Only 2 factors affect alveolar PCO_2: metabolic rate and alveolar ventilation.

$$PACO_2 \propto \frac{\text{metabolic } CO_2 \text{ production}}{\text{alveolar ventilation}}$$

At rest, unless there is fever or hypothermia, CO_2 production is relatively constant; so you can use changes of $PACO_2$ to evaluate alveolar ventilation.

Alveolar Ventilation

There is an inverse relationship between $PACO_2$ and alveolar ventilation. This is the main factor affecting alveolar PCO_2. Therefore, if ventilation increases, $PACO_2$ decreases; if ventilation decreases, $PACO_2$ increases.

Hyperventilation

During hyperventilation, there is an inappropriately elevated level of alveolar ventilation, and $PACO_2$ is depressed.

If $\dot{V}A$ is doubled, then $PACO_2$ is decreased by half.

For example, $PACO_2 = 40$ mm Hg

$2 \times \dot{V}A$; $PACO_2 = 20$ mm Hg

Hypoventilation

During hypoventilation, there is an inappropriately depressed level of alveolar ventilation, and $PACO_2$ is elevated.

If $\dot{V}A$ is halved, then $PACO_2$ is doubled.

For example, $PACO_2 = 40$ mm Hg

$1/2\ \dot{V}A$; $PACO_2 = 80$ mm Hg

Metabolic Rate

There is a direct relationship between alveolar PCO_2 and body metabolism. For $PaCO_2$ to remain constant, changes in body metabolism must be matched with equivalent changes in alveolar ventilation.

- If $\dot{V}A$ matches metabolism, then $PACO_2$ remains constant.
- For example, during exercise, if body metabolism doubles, then $\dot{V}A$ must double if $PaCO_2$ is to remain constant.
- If body temperature decreases and there is no change in ventilation, $PaCO_2$ decreases, and the individual can be considered to be hyperventilating.

FACTORS AFFECTING ALVEOLAR PO_2

Alveolar Air Equation

The alveolar air equation includes all the factors that can affect alveolar PO_2.

$$PAO_2 = (Patm - 47)FiO_2 - \frac{PACO_2}{RQ}$$

Practical application of the equation includes differential diagnosis of hypoxemia by evaluating the alveolar arterial (A–a) gradient of oxygen.

There are 3 factors that can affect PAO_2:

Patm = atmospheric pressure, at sea level 760 mm Hg

An increase in atmospheric pressure (hyperbaric chamber) increases alveolar PO_2, and a decrease (high altitude) decreases alveolar PO_2.

FiO_2 = fractional concentration of oxygen, room air 0.21

Note

Respiratory quotient (RQ) is the ratio between CO_2 production and O_2 consumption at the cellular level. **Respiratory exchange ratio (RER)** is the ratio of CO_2 output and oxygen uptake occurring in the lung.

In a steady state, RQ and RER are equal.

An increase in inspired oxygen concentration increases alveolar PO_2.

$PaCO_2$ = alveolar pressure of carbon dioxide, normally 40 mm Hg

An increase in alveolar PCO_2 decreases alveolar PO_2, and a decrease in alveolar PCO_2 increases alveolar PO_2. For most purposes, you can use arterial carbon dioxide ($PaCO_2$) in the calculation.

The fourth variable is RQ.

$$RQ = \text{respiratory exchange ratio} = \frac{CO_2 \text{ produced mL/min}}{O_2 \text{ consumed mL/min}}; \text{ normally } 0.8$$

For example, a person breathing room air at sea level would have

$PAO_2 = (760 - 47)\ 0.21 - 40/0.8 = 100$ mm Hg.

Effect of $PACO_2$ on PAO_2

PIO_2 = P inspired O_2, i.e., the PO_2 in the conducting airways during inspiration.

Because $PaCO_2$ affects alveolar PO_2, hyperventilation and hypoventilation also affect PaO_2.

Hyperventilation (e.g., $PaCO_2$ = 20 mm Hg)

$PaO_2 = PiO_2 - PaCO_2$ (assume R = 1)
normal = 150 – 40 = 110 mm Hg
hyperventilation = 150 – 20 = 130 mm Hg

Hypoventilation (e.g., $PaCO_2$ = 80 mm Hg)

normal = 150 – 40 = 110 mm Hg
hypoventilation = 150 – 80 = 70 mm Hg

ALVEOLAR–BLOOD GAS TRANSFER: FICK LAW OF DIFFUSION

Simple diffusion is the process of gas exchange between the alveolar compartment and pulmonary capillary blood. Thus, those factors that affect the rate of diffusion also affect the rate of exchange of O_2 and CO_2 across alveolar membranes. (An additional point to remember is that each gas diffuses independently.)

$\dot{V}gas$ = rate of gas diffusion

$$\dot{V}gas = \frac{A}{T} \times D \times (P_1 - P_2)$$

Structural Features That Affect the Rate of Diffusion

There are 2 structural factors and 2 gas factors affect the rate of diffusion.

A = surface area for exchange, ↓ in emphysema, ↑ in exercise

T = thickness of the membranes between alveolar gas and capillary blood, ↑ in fibrosis and many other restrictive diseases

A structural problem in the lungs is any situation in which there is a loss of surface area and/or an increase in the thickness of the membrane system between the alveolar air and the pulmonary capillary blood. In all cases, the rate of oxygen and carbon dioxide diffusion decreases. The greater the structural problem, the greater the effect on diffusion rate.

Factors Specific to Each Gas Present

D (diffusion constant) = main factor is solubility

The only clinically significant feature of D is solubility. The more soluble the gas, the faster it diffuses across the membranes. CO_2 is the most soluble gas with which we will be dealing. The great solubility of CO_2 is the main reason why it diffuses faster across the alveolar membranes than O_2.

Gradient across the membrane

($P_1 - P_2$): This is the gas partial pressure difference across the alveolar membrane. The greater the partial pressure difference, the greater the rate of diffusion.

Under resting conditions, when blood first enters the pulmonary capillary, the gradient for O_2 is:

$$100 - 40 = 60 \text{ mm Hg}$$

An increase in the PO_2 gradient across the lung membranes helps compensate for a structural problem. If supplemental O_2 is administered, alveolar PO_2 increases, because of the elevated gradient. However, supplemental O_2 does not improve the ability of the lungs to remove CO_2 from blood. This increased gradient helps return the rate of O_2 diffusion toward normal. The greater the structural problem, the greater the gradient necessary for a normal rate of O_2 diffusion.

The gradient for CO_2 is $47 - 40 = 7$ mm Hg.

Even though the gradient for CO_2 is less than for O_2, CO_2 still diffuses faster because of its greater solubility.

Recall Question

Which of the following factors increases alveolar PCO_2, assuming no compensation?

A. Decrease in atmospheric pressure (Patm)

B. Increase in fractional concentration of oxygen (FiO_2)

C. Decrease in compliance of alveoli

D. Increase in thickness of the membranes between alveolar gas and capillary blood

E. Increase in body temperature

Answer: E

DIFFUSING CAPACITY OF THE LUNG

There are 2 terms that describe the dynamics of the transfer of individual substances between the interstitium and the capillary:

- If the substance equilibrates between the capillary and interstitium, it is said to be in a **perfusion-limited situation**.
- If the substance does not equilibrate between the capillary and interstitium, it is said to be in a **diffusion-limited situation**.

Carbon monoxide is a unique gas in that it typically doesn't equilibrate between the alveolar air and the capillary blood. Thus, it is a diffusion-limited gas. This is taken advantage of clinically, and the measurement of the uptake of CO in mL/min/mm Hg is referred to as the diffusing capacity of the lung (DLCO).

DLCO is an index of the lung's structural features.

Carbon Monoxide: A Gas That Is Always Diffusion Limited

Carbon monoxide has an extremely high affinity for hemoglobin. When it is present in the blood, it rapidly combines with hemoglobin, and the amount dissolved in the plasma is close to zero (therefore, partial pressure in the plasma is considered zero). Thus, the alveolar partial pressure gradient ($P_1 - P_2$) is simply P_1 (alveolar partial pressure), since P_2 is considered to be zero.

At a constant and known alveolar partial pressure, the uptake of carbon monoxide depends only on the structural features of the lung.

$$\dot{V}\text{gas} = \frac{A}{T} \times D \times (P_1 - P_2)$$

$$\dot{V}CO = \frac{A}{T} \times D \times P_A CO$$

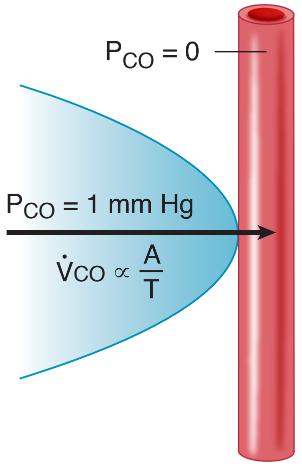


Figure V-2-2. Carbon Monoxide

This measured uptake of carbon monoxide is called the diffusing capacity of the lung (DL; mL/min/mm Hg). It is an index of overall surface area and membrane thickness.

- With a structural problem, it correlates with the extent of lung damage and is particularly useful when measured serially over time.
- DL (rate of CO diffusion) decreases in emphysema and fibrosis but increases during exercise.

Transport of O_2 and CO_2 and the Regulation of Ventilation

3

Learning Objectives

- ❑ Interpret scenarios on transport of oxygen
- ❑ Answer questions about transport of carbon dioxide
- ❑ Interpret scenarios on neural regulation of alveolar ventilation
- ❑ Answer questions about respiratory stress: unusual environments

TRANSPORT OF OXYGEN

Units of Oxygen Content

Oxygen content = concentration of oxygen in the blood, e.g., arterial blood
= 20 volumes %
= 20 volumes of oxygen per 100 volumes of blood
= 20 mL of oxygen per 100 mL of blood
= 0.2 mL of oxygen per mL of blood

Dissolved Oxygen

Oxygen dissolves in blood and this dissolved oxygen exerts a pressure. Thus, PO_2 of the blood represents the pressure exerted by the dissolved gas, and this PO_2 is directly related to the amount dissolved.

The amount dissolved (PO_2) is the primary determinant for the amount of oxygen bound to hemoglobin (Hb).

There is a direct linear relationship between PO_2 and dissolved oxygen.

- When PO_2 is 100 mm Hg, 0.3 mL O_2 is dissolved in each 100 mL of blood (0.3 vol%).
- Maximal hyperventilation can increase the PO_2 in blood to 130 mm Hg (0.4 vol%).

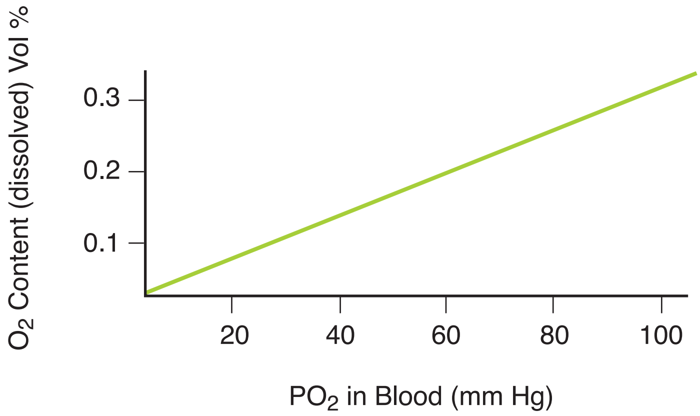


Figure V-3-1. Dissolved Oxygen in Plasma

Oxyhemoglobin

Each Hb molecule can attach and carry up to four oxygen molecules. Binding sites on Hb have different affinities for oxygen. Also, the affinity of a site can and does change as oxygen is loaded or unloaded from the Hb molecule and as the chemical composition of the plasma changes.

Site 4 – O_2 attached when the minimal $PO_2 \cong 100$ mm Hg	systemic arterial blood = 97% saturated
Site 3 – O_2 attached when the minimal $PO_2 \cong 40$ mm Hg	systemic venous blood = 75% saturated (resting state)
Site 2 – O_2 attached when the minimal $PO_2 \cong 26$ mm Hg	P_{50} for arterial blood. P_{50} is the PO_2 required for 50% saturation
Site 1 – O_2 usually remains attached under physiologic conditions.	Under physiologic conditions, only sites 2, 3, and 4 need to be considered.

Most of the oxygen in systemic arterial blood is oxygen attached to Hb. The only significant form in which oxygen is delivered to systemic capillaries is oxygen bound to Hb.

Hemoglobin O_2 Content

The number of mL of oxygen carried in each 100 mL of blood in combination with Hb depends on the Hb concentration [Hb]. Each gram of Hb can combine with 1.34 mL of O_2.

If the [Hb] is 15 g/100 mL (15 g%), then the maximal amount of O_2 per 100 mL (100% saturation) in combination with Hb is:

$1.34([Hb]) = 1.34(15) = 20$ mL O_2/100 mL blood = 20 vol%

This volume represents the "carrying capacity" of the blood.

The Hb in systemic arterial blood is about 97% saturated with oxygen, which means slightly less than 20 vol% is carried by Hb.

When blood passes through a systemic capillary, it is the dissolved oxygen that diffuses to the tissues. However, if dissolved oxygen decreases, PO_2 also decreases, and there is less force to keep oxygen attached to Hb. Oxygen comes off Hb and dissolves in the plasma to maintain the flow of oxygen to the tissues.

Hyperventilation or supplementing the inspired air with additional oxygen in a normal individual can significantly increase the PaO_2 but has little effect on total oxygen content. For example:

	Dissolved O_2	HbO_2	Total O_2 Content
If $PaO_2 = 100$ mm Hg	0.3	≅ 19.4	≅ 19.7 vol%
If $PaO_2 = 130$ mm Hg (hyperventilation)	0.4	≅ 19.4	≅ 19.8 vol%

Oxygen–Hb Dissociation Curves

The figure below represents 3 major points on the oxygen–hemoglobin dissociation curve. The numbered sites refer to the hemoglobin site numbers discussed just previously.

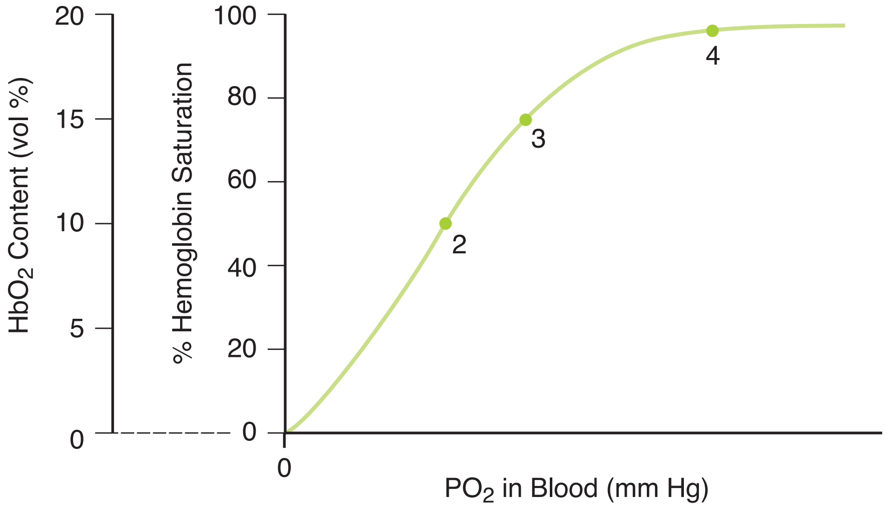


Figure V-3-2. Oxygen–Hb Dissociation Curves

The following factors **shift the curve to the right**:

- Increased CO_2 (Bohr effect)
- Increased hydrogen ion (decrease pH)
- Increased temperature
- Increased 2,3-bisphosphoglycerate (2,3-BPG)

In each case, the result can be explained as a reduced affinity of the Hb molecule for oxygen. However, carrying capacity is not changed, and systemic arterial blood at a PO_2 of 100 mm Hg is still close to 100% saturation.

Note that only points on the steep part of the curve are affected.

The opposite chemical changes **shift the curve to the left**.

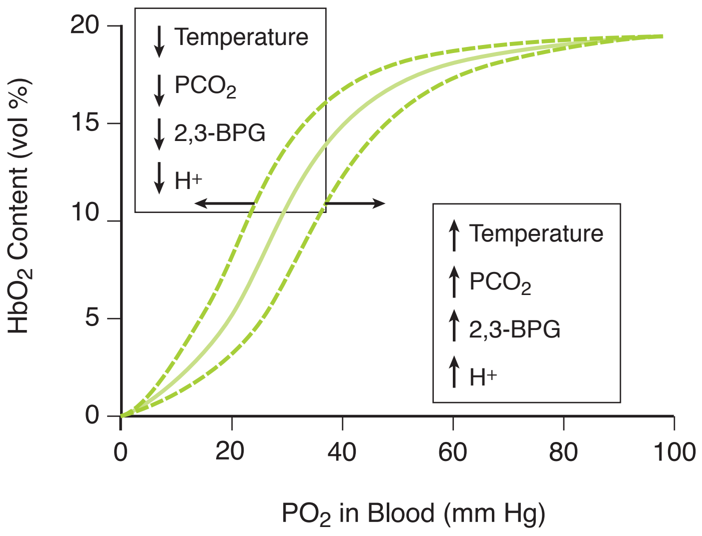


Figure V-3-3. Shifts in Hb–O_2 Dissociation Curve

Shift to the Right	Shift to the Left
Easier for tissues to extract oxygen	More difficult for tissues to extract oxygen
Steep part of curve, O_2 content decreased	Steep part of curve, O_2 content increased
P_{50} increased	P_{50} decreased

Stored blood loses 2,3-bisphosphoglycerate, causing a left shift in the curve, while hypoxia stimulates the production of 2,3-bisphosphoglycerate, thereby causing a right shift.

Hb Concentration Effects

Anemia is characterized by a reduced concentration of Hb in the blood.

Polycythemia is characterized by a higher than normal concentration of Hb in the blood.

P_{50}: In simple anemia and polycythemia, the P_{50} does not change without tissue hypoxia; e.g., PO_2 of 26 mm Hg produces 50% saturation of arterial hemoglobin.

The figure below illustrates the effects of an increase and a decrease in hemoglobin concentration. The main change is the plateau or carrying capacity of the blood.

Note that the point halfway up each curve, the P_{50}, is still close to 26 mm Hg.

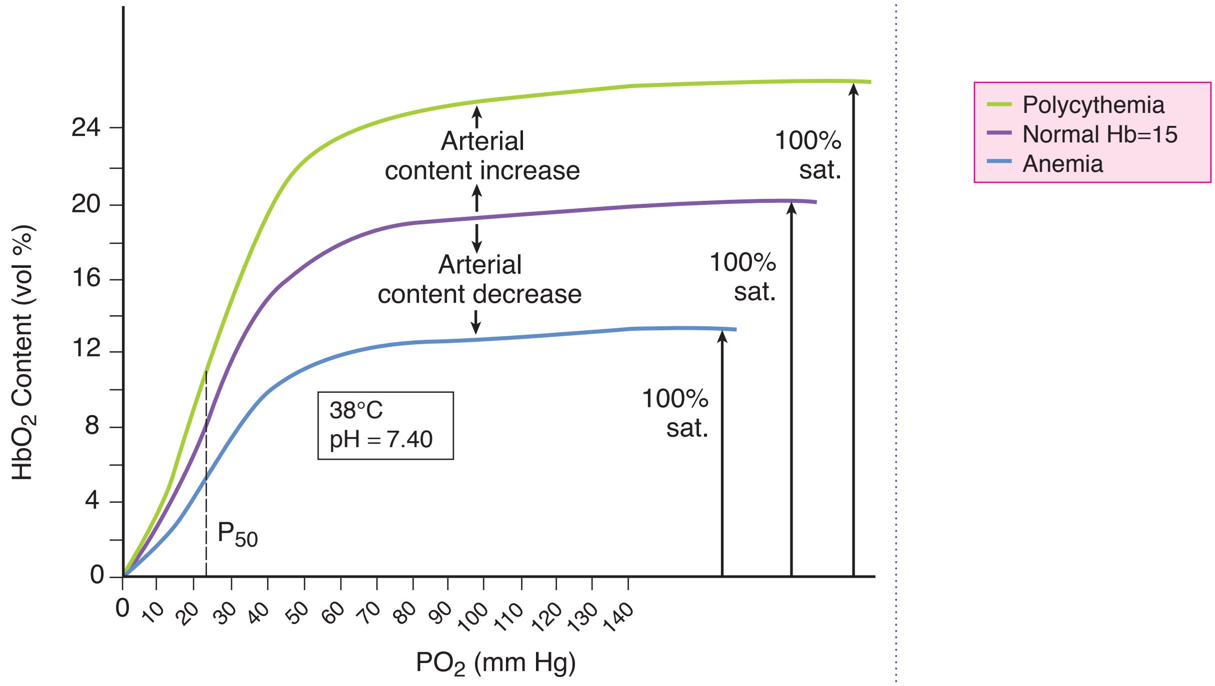


Figure V-3-4. Effect of Hemoglobin Content on O_2 Content

Effects of Carbon Monoxide

Carbon monoxide (CO) has a greater affinity for Hb than does oxygen (240x greater). The figure below shows that with CO, the O_2–Hb dissociation curve is shifted to the left (CO increases the affinity of Hb for O_2) and HbO_2 content is reduced.

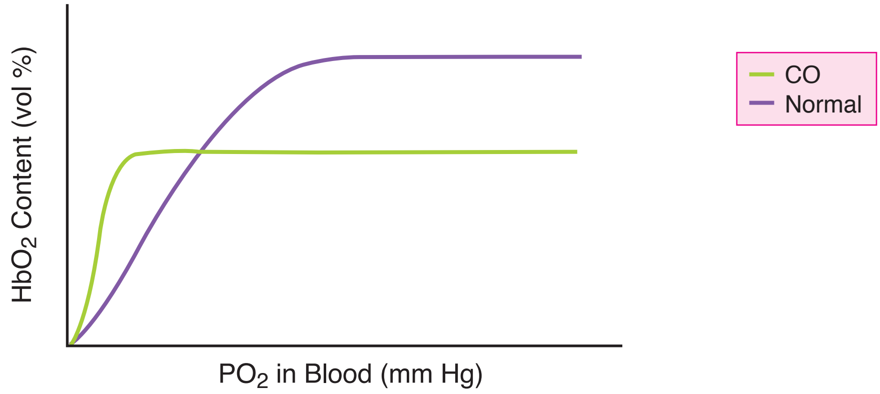


Figure V-3-5. Carbon Monoxide Poisoning

The effects of anemia, polycythemia, and carbon monoxide poisoning are summarized below.

Table V-3-1. Systemic Arterial Blood

	PO_2	Hb Concentration	O_2 per g Hb	O_2 Content
Anemia	Normal	↓	Normal	↓
Polycythemia	Normal	↑	Normal	↑
CO poisoning (acute)	Normal	Normal	↓	↓

O_2 per g Hb = % saturation

In **anemia**, hemoglobin is saturated but arterial oxygen content is depressed because of the reduced concentration of hemoglobin.

In **polycythemia**, arterial oxygen content is above normal because of an increased hemoglobin concentration.

In **CO poisoning**, arterial PO_2 is normal, but oxygen saturation of hemoglobin is depressed.

TRANSPORT OF CARBON DIOXIDE

Dissolved Carbon Dioxide

Carbon dioxide is 24x more soluble in blood than oxygen is. Even though the blood has a PCO_2 of only 40–47 mm Hg, about 5% of the total CO_2 is carried in the dissolved form.

Carbamino Compounds

Carbon dioxide reacts with terminal amine groups of proteins to form carbamino compounds. The protein involved appears to be almost exclusively hemoglobin. About 5% of the total CO_2 is carried as carbamino compounds. The attachment sites that bind CO_2 are different from the sites that bind O_2.

Bicarbonate

About 90% of the CO_2 is carried as plasma bicarbonate. In order to convert CO_2 into bicarbonate or the reverse, carbonic anhydrase (CA) must be present.

$$CO_2 + H_2O \overset{CA}{\leftrightarrow} H_2CO_3 \leftrightarrow H^+ + HCO_3-$$

The steps in the conversion of CO_2 into bicarbonate in a systemic capillary are seen below.

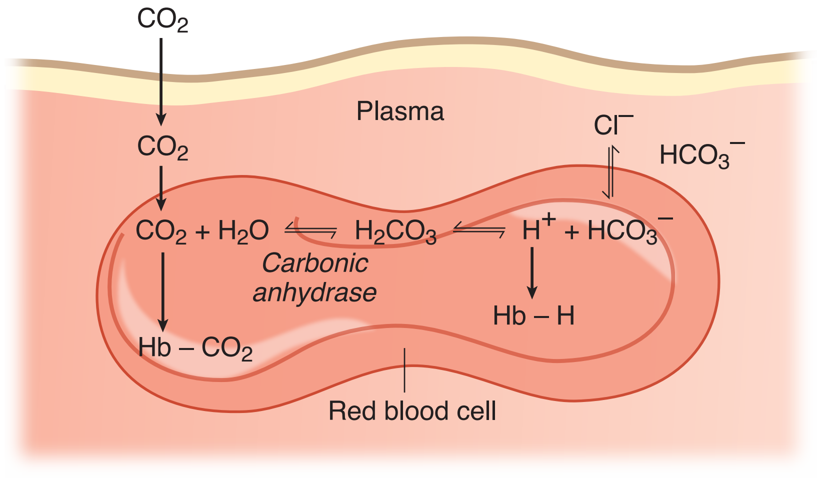


Figure V-3-6. Formation of Bicarbonate Ion

Plasma contains no carbonic anhydrase; therefore, there can be no significant conversion of CO_2 to HCO_3^- in this compartment.

Because deoxygenated Hb is a better buffer, removing oxygen from hemoglobin shifts the reaction to the right and thus facilitates the formation of bicarbonate in the red blood cells (Haldane effect).

To maintain electrical neutrality as HCO_3^- moves into the plasma, Cl^- moves into the red blood cell (chloride shift).

In summary:

- Bicarbonate is formed in the red blood cell but it is carried in the plasma compartment.
- The PCO_2 determines the volume of CO_2 carried in each of the forms listed above. The relationship between the PCO_2 and the total CO_2 content is direct and nearly linear.
- Thus, hyperventilation not only lowers the PCO_2 (mm Hg), it also lowers the CO_2 content (vol%).

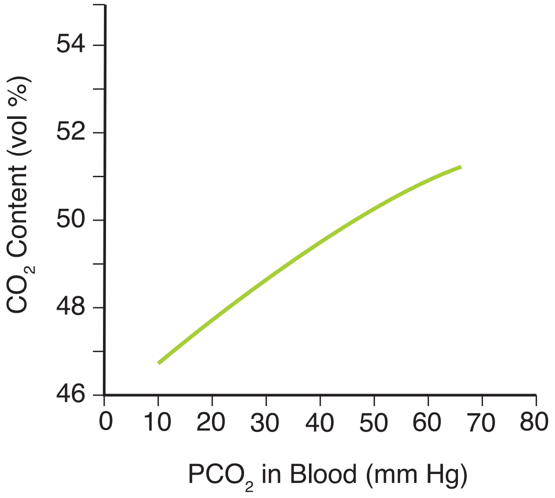


Figure V-3-7. CO_2 Content in Blood

NEURAL REGULATION OF ALVEOLAR VENTILATION

The level of alveolar ventilation is driven mainly from the input of specific chemoreceptors to the central nervous system. The stronger the stimulation of these receptors, the greater the level of alveolar ventilation. Chemoreceptors monitor the chemical composition of body fluids. In this system, there are receptors that respond to pH, PCO_2, and PO_2.

There are 2 groups of receptors, and they are classified by their location.

Central Chemoreceptors

Central receptors are located in the central nervous system—more specifically, close to the surface of the medulla. Stimulation of central chemoreceptors increases ventilation.

- The receptors directly monitor and are stimulated by cerebrospinal fluid $[H^+]$ and CO_2. The stimulatory effect of increased CO_2 may be due to the local production of H^+ from CO_2.
- Because the blood–brain barrier is freely permeable to CO_2, the activity of these receptors changes with increased or decreased systemic arterial PCO_2.
- H^+ does not easily penetrate the blood-brain barrier. Thus, an acute rise in arterial H^+, not of CO_2 origin, does not stimulate central chemoreceptors.
- These receptors are very sensitive and represent the main drive for ventilation under normal resting conditions at sea level.
- Therefore, the main drive for ventilation is CO_2 (H^+) on the central chemoreceptors.

The relationship between the central chemoreceptors and systemic arterial blood can be seen below.

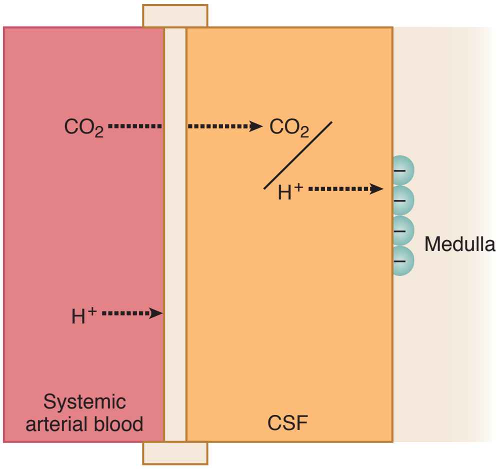


Figure V-3-8. Central Chemoreceptors

The system does adapt, usually within 12–24 hours. The mechanism of adaptation may be the normalization of CSF H^+ by the pumping of HCO_3^- into or out of the CSF. There are no central PO_2 receptors.

Peripheral Chemoreceptors

Peripheral receptors are found within small bodies at 2 locations:

- **Carotid bodies:** near carotid sinus, afferents to CNS in glossopharyngeal nerve IX
- **Aortic bodies:** near aortic arch, afferents to CNS in vagus nerve X

The peripheral chemoreceptors are bathed in arterial blood, which they monitor directly. These bodies have 2 different receptors:

- **H^+/CO_2 receptors**
  - These receptors are less sensitive than the central chemoreceptors, but they still contribute to the normal drive for ventilation.
  - Therefore, under normal resting conditions at sea level, for all practical purposes, the total drive for ventilation is CO_2, mainly via the central chemoreceptors but with a small contribution via the peripheral chemoreceptors.
- **PO_2 receptors**
  - The factor monitored by these receptors is PO_2, not oxygen content.
  - Because they respond to PO_2, they are actually monitoring dissolved oxygen and not oxygen on Hb.
  - When systemic arterial PO_2 is close to normal (≅100 mm Hg) or above normal, there is little if any stimulation of these receptors.
- They are strongly stimulated only by a dramatic decrease in systemic arterial PO_2.
- Sensitivity to hypoxia increases with CO_2 retention.

These receptors do not adapt.

Bridge to Pathology/ Pharmacology

The normal CO_2 drive to breathe is suppressed in COPD patients, and by narcotics and general anesthetics.

Clinical Correlate

Although oxygen content is reduced in anemia, the PaO_2 is normal; thus, anemia does not directly stimulate ventilation. However, the reduced oxygen delivery can cause excess lactic acid production, which would in turn stimulate peripheral chemoreceptors.

Central Respiratory Centers

Medullary centers

Site of the inherent rhythm for respiration.

Inspiratory center

Expiratory center

For spontaneous breathing, an intact medulla must be connected to the diaphragm (via the phrenic nerve). Thus a complete C1 or C2 lesion will prevent diaphragmatic breathing but not a complete C6 or lower lesion.

The main features involved in the central control of ventilation are seen below.

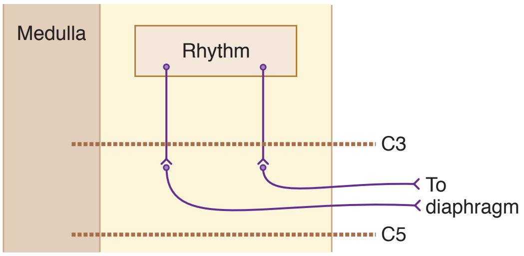


Figure V-3-9. CNS Respiratory Centers

Abnormal Breathing Patterns

Apneustic breathing is prolonged inspirations alternating with a short period of expiration. This pattern is attributed to the loss of the normal balance between vagal input and the pons-medullary interactions. Lesions in these patients are usually found in the caudal pons.

Cheyne-Stokes breathing is periodic type of breathing which has cycles of gradually increasing depth and frequency followed by a gradual decrease in depth and frequency between periods of apnea. It may result from midbrain lesions or congestive heart failure.

RESPIRATORY STRESS: UNUSUAL ENVIRONMENTS

High Altitude

At high altitude, atmospheric pressure is reduced from 760 mm Hg of sea level. Because atmospheric pressure is a factor that determines room air and alveolar PO_2, those 2 values are also reduced; they are permanently depressed unless enriched oxygen is inspired.

Therefore, PAO_2 <100 mm Hg, PaO_2 <100 mm Hg, and the low arterial PO_2 stimulates the peripheral chemoreceptors and increases alveolar ventilation. At high altitude, then, the main drive for ventilation changes from CO_2 on the central chemoreceptors at sea level to a low PO_2 drive of the peripheral chemoreceptors, and hyperventilation ensues.

Table V-3-2. Acute Changes and Long-Term Adaptations (Acclimatization)

	Acute Changes	Acclimatization
PAO_2 and PaO_2	decreased	remains decreased
$PACO_2$ and $PaCO_2$	decreased	remains decreased
Systemic arterial pH	increased	decreases to normal via renal compensation
Hb concentration	no change	increases (polycythemia)
Hb % sat	decreased	remains decreased
Systemic arterial O_2 content	decreased	increases to normal

At high altitude, hypoxia can develop, resulting in increased circulating levels of erythropoietin and red cell concentration of 2,3-bisphosphoglycerate (right shifts the oxygen-hemoglobin dissociation curve). Erythropoietin increases red blood cell production and eventually causes an adaptive polycythemia.

High-Pressure Environment

In a hyperbaric environment breathing room air (21% O_2 and 79% N_2), the partial pressure of O_2 and N_2 increase in the alveoli and systemic arterial blood. The pressure of nitrogen also increases in other body compartments.

Oxygen

- Adverse effect is oxygen toxicity due to the production of oxygen radicals.
- Clinical uses include carbon monoxide poisoning, compromised tissue grafts, and gas gangrene.

Nitrogen

- **Rapture of the deep:** a feeling of euphoria associated with high nitrogen levels
- **The bends** (Caisson's disease, or decompression sickness) too-rapid decompression after exposure to high nitrogen pressures. It can result in nitrogen coming out of solution in joints (bends) or in the blood, resulting in air emboli in the vasculature.

Recall Question

Which of the following factors causes a left shift on the oxygen-hemoglobin dissociation curve?

A. Increased CO_2

B. Metabolic acidosis

C. Temperature 104° F

D. Decreased 2,3 BPG

E. Respiratory acidosis

Answer: D

Note

What principle explains the physiology of why nitrogen will be forced into solution?

Answer: Henry's law. The amount of gas that will dissolve in a liquid varies directly with the pressure above that liquid. High pressures force gas into solution. However, solubilities and temperature also come into play when considering Henry's law. Even though a huge N_2 gradient may exist between the air and plasma, nitrogen is barely soluble at all.

Bridge to Microbiology

Gas gangrene is caused by the bacteria *Clostridium perfringens*. This bacteria thrives in an anaerobic environment, explaining why hyperbaric oxygen can be helpful. *Staphylococcus aureus* and *Vibrio vulnificus* can cause similar infections.

Ventilation/Perfusion Matching and Hypoxemia

4

Learning Objectives

- ❑ Demonstrate understanding of ventilation-perfusion differences in the lung
- ❑ Demonstrate understanding of review of the normal lung
- ❑ Answer questions about causes of hypoxemia
- ❑ Use knowledge of left-to-right shunts

VENTILATION/PERFUSION DIFFERENCES IN THE LUNG

Regional Differences in Intrapleural Pressure (IPP)

At FRC, the mean value for intrapleural pressure is –5 cm H_2O. However, there are regional differences, and the reason for these differences is gravity.

- Recall that the pleura is a fluid-filled space.
- Similar to the cardiovascular system, it is subject to gravitational influences
  - (P = height × gravity × density)
- Thus, IPP is higher (less negative) at the base (bottom) of the lung compared to the apex (top).

Clinical Correlate

The regional difference of alveolar and arterial pressure in the lung is referred to as “west zones” of the lung. The point is that the ventilation perfusion ratio is higher in the apex of the lung (zone 1) in an upright individual than it is in the base of the lung (zone 3).

Regional Difference in Ventilation

- Because IPP is higher (less negative) at the base, the P_{TM} is less, resulting in less distension of alveoli, i.e., there is less volume.
- In contrast, IPP is more negative at the apex, thus the P_{TM} is higher, resulting in a greater volume in alveoli near the apex.
- As described in chapter 1, alveolar compliance decreases as lung volume increases. Thus, alveoli near the base are more compliant than alveoli near the apex. Stated another way, alveoli near the base are on a much steeper portion of the pressure-volume curve than alveoli near the apex (Figure V-4-2).
- Because alveoli near the base are more compliant, there is more ventilation in this region compared to the apex.

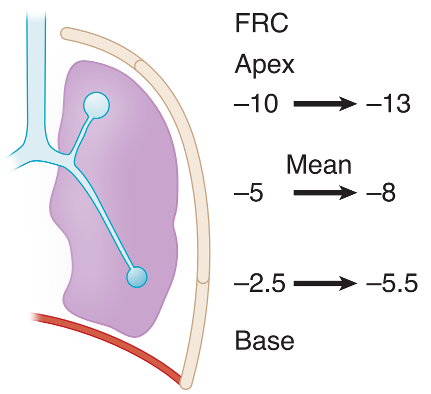


Figure V-4-1. Upright Posture

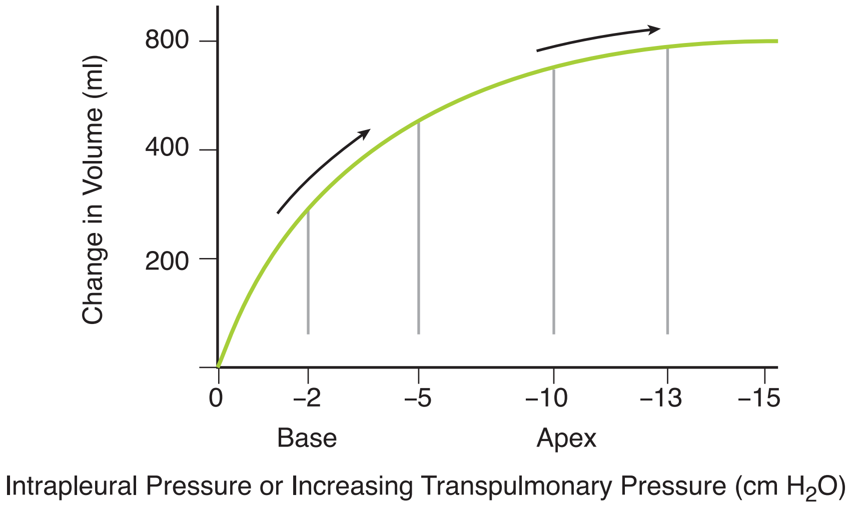


Figure V-4-2. Regional Ventilation

Regional Differences in Blood Flow

Even in a normal individual, there are regional differences in blood flow through the pulmonary circuit. These differences, for the most part, can be attributed to the effect of gravity.

- Moving toward the base (with gravity), pressure in the pulmonary arteries is higher compared to pressure in the pulmonary arteries of the apex (against gravity).
- Since the intravascular pressure in arteries is higher, there is more blood flow to the base of the lung compared to the apex.

Ventilation–Perfusion Relationships

The partial pressures of O_2 and CO_2 in alveoli are determined by the combination of ventilation (adding O_2, removing CO_2) and perfusion (removing O_2 and adding CO_2). However, it is not the absolute amount of either that determines the composition of alveolar gases. Instead, it is the relative relationship between ventilation and perfusion that ultimately determines the alveolar gases. This is **ventilation-perfusion matching.**

In the normal situation, it would be "ideal" if ventilation and perfusion (blood flow) matched, i.e., the ventilation-perfusion ratio is one (Figure V-4-3). If this were the case, then:

- $PaO_2 = 100$ mm Hg
- $PaCO_2 = 40$ mm Hg
- The blood draining the alveolus would have a pH = 7.40 (normal blood pH)

Although the above is "ideal," it is not often encountered. The figure below illustrates ventilation, blood flow (Q) or perfusion, and the relative ventilation-perfusion relationship for an **upright individual**. Toward the base of the lung:

- Alveolar ventilation is high relative to the apex (described above).
- Q is high relative to the apex (described above).
- However, relative to one another, Q is higher than alveolar ventilation, thus the ventilation-perfusion relationship is <1.0.
- In short, the alveoli are under-ventilated relative to the perfusion. If alveolar ventilation is inadequate, then it follows that PO_2 falls, PCO_2 rises, and blood pH falls (remember that CO_2 generates H^+).
- Thus, PaO_2 at the base is <100 mm Hg and $PaCO_2$ is >40 mm Hg.

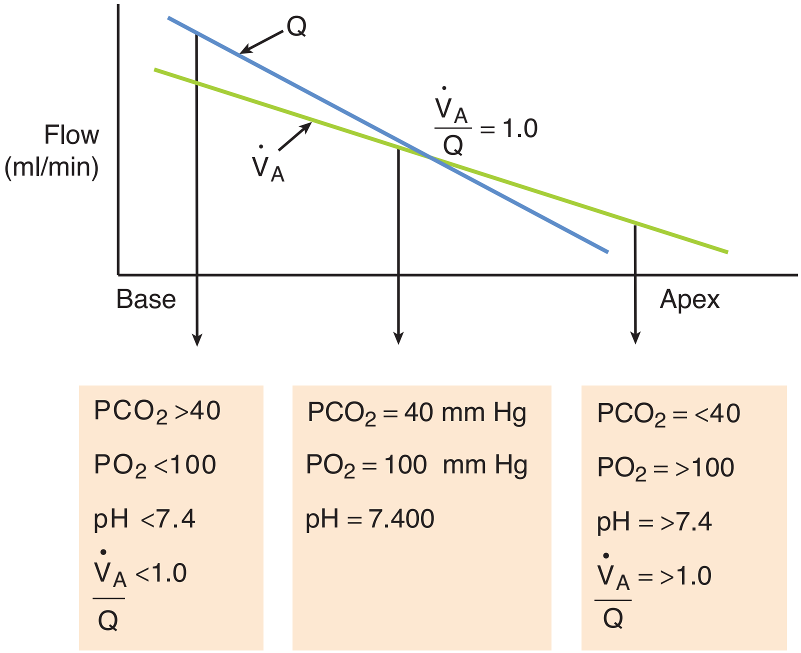


Figure V-4-3. Ventilation–Perfusion Relationships

Moving toward the apex, the situation reverses:

- Alveolar ventilation is less relative to the base (described above).
- Q is less relative to the base (described above).
- However, relative to one another, Q is less than alveolar ventilation, thus the ventilation-perfusion relationship is >1.0.
- In short, the alveoli are over-ventilated relative to the perfusion. If alveolar ventilation is excessive, then it follows that PO_2 rises, PCO_2 falls, and blood pH increases (remember that CO_2 generates H^+).
- Thus, PAO_2 at the apex is >100 mm Hg and $PACO_2$ is <40 mm Hg.

The effect of the ventilation-perfusion relationship is a continuum.

- As **$\dot{V}$A/Q falls**, PO_2 falls and PCO_2 rises.
- As **$\dot{V}$A/Q rises**, PO_2 rises and PCO_2 falls.

Extremes of $\dot{V}A/Q$ Mismatch

- **Shunt:** If ventilation is zero but there is blood flow, then $\dot{V}A/Q = 0$.
  - This is a right-to-left shunt, and the blood gases leaving the alveoli are the same as venous blood (low PO_2, and high PCO_2; Y-axis intercept in figure below). This causes arterial hypoxemia, which is discussed later in this chapter.
- **Alveoline dead space:** If blood flow is zero but there is ventilation, then $\dot{V}A/Q = \infty$.
  - This is alveolar dead space, and alveolar gases become the same as inspired (high PaO_2 and $PaCO_2 = 0$; X-axis intercept in figure below).

To summarize:

- As $\dot{V}A/Q$ falls, PO_2 falls and PCO_2 rises. The extreme is a shunt.
  - Remember, however, that the lower the $\dot{V}A/Q$, the more it "behaves" as a shunt, i.e., the alveolar and blood gases get closer and closer to venous gases. Similar to a shunt, this can lead to arterial hypoxemia, both of which are discussed later in this chapter.
- As $\dot{V}A/Q$ rises, PO_2 rises and PCO_2 falls. The extreme is alveolar dead space.
  - Similar to above, the higher the $\dot{V}_A/Q$, the more the situation looks like alveolar dead space.

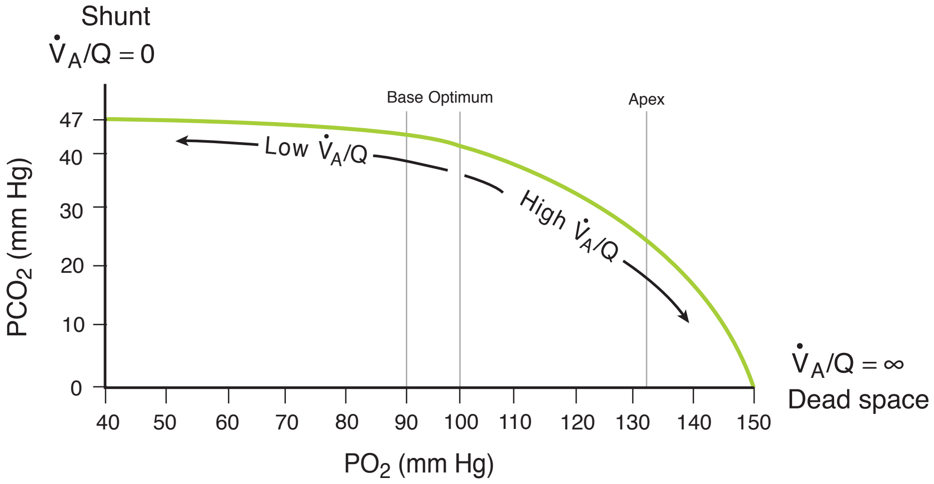


Figure V-4-4. Shunt and Dead Space

Problem

The following ratios represent 2 lung units under resting conditions:

$\dot{V}A/Q$

$A = 0.62$

$B = 0.73$

Both lung units A and B are underventilated, but of the two, B is better ventilated.

Which lung unit had the greatest:

$PACO_2$, end capillary PCO_2? (Answer: A)

PAO_2, end capillary PO_2? (Answer: B)

end capillary pH? (Answer: B)

Hypoxic Vasoconstriction

This is a clinically important phenomenon that is unique to the pulmonary circulation. Whenever there is a decrease in alveolar PO_2, a local vasoconstriction of pulmonary blood vessels is produced. The result is a lowering of blood flow through that lung unit and a redistribution of blood to better-ventilated units.

Problem

If a person inhales a peanut that lodges in a peripheral airway, what changes would you expect for the following variables in the peanut-occluded unit?

$PACO_2$ (increase)

PAO_2 (decrease)

pulmonary end capillary pH (decrease)

blood flow in that lung unit (decrease)

All answers here are based on the fact that blocking the airway produces a shunt. The blood flow decreases because of hypoxic vasoconstriction. Low $\dot{V}A$ /Q ratios are associated with hypoxic vasoconstriction. If the pulmonary disease is severe and widespread, the alveolar hypoxia and subsequent arteriolar vasoconstriction increases pulmonary arterial pressure.

Problem

If a small thrombus lodges in a pulmonary artery, what changes would you expect for the following variables in the thrombus-occluded unit?

$PACO_2$ (decrease)

PAO_2 (increase)

pulmonary end capillary pH (increase)

All answers here are based on the fact that the thrombus increases the $\dot{V}A$/Q ratio. This produces lung units that act as dead space.

Exercise

In exercise, there is increased ventilation and pulmonary blood flow. However, during exercise, ventilation increases more than cardiac output and $\dot{V}A$/Q goes well above 1.0 as one approaches maximal oxygen consumption. Also, the base–apex flows are more uniform.

Clinical Correlate

As one ages, the A–a gradient increases because ventilation-perfusion matching becomes less and less "ideal."

One formula for taking this into account is:

$$\frac{(\text{age} + 4)}{4}$$

REVIEW OF THE NORMAL LUNG

Before discussing the causes of hypoxemia let's review the normal state using standard values:

- The blood entering the alveolar-capillary unit is mixed venous blood.
  - $PO_2 = 40$ and $PCO_2 = 45$ mm Hg
- $PaO_2 = 100$ mm Hg and $PaCO_2 = 40$ mm Hg
- Both gases are perfusion-limited and thus their partial pressures at the end of the capillary are the same as alveolar.
- Arterial blood gas (ABG) sample shows $PaO_2 = 95$ mm Hg, and $PaCO_2 = 40$ mm Hg.
  - The A–a gradient is 5 mm Hg (ranges 5-10 mm Hg but is influenced by age) and is primarily the result of anatomic shunts.

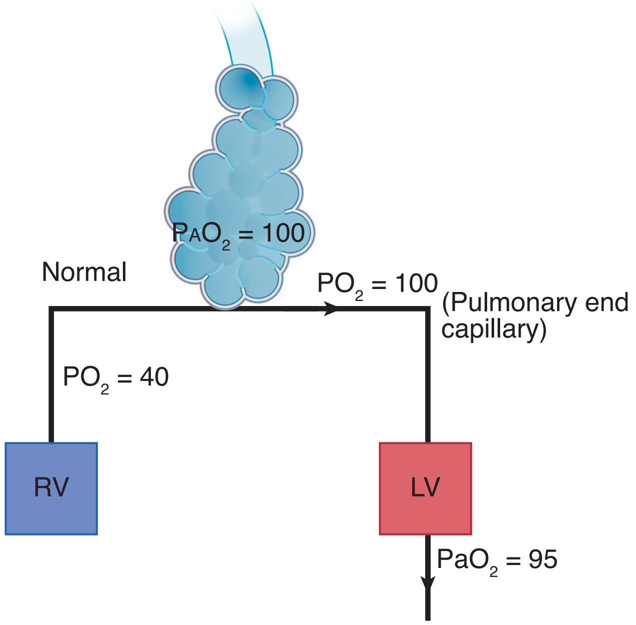


Figure V-4-5. Normal State

CAUSES OF HYPOXEMIA

Hypoventilation

Hypoventilation of the entire lung elevates alveolar PCO_2, and the increase in PCO_2 decreases PO_2. For example, if alveolar ventilation decreases by 50%, alveolar PCO_2 becomes 80 mm Hg (an increase of 40 mm Hg).

Assuming a respiratory ratio close to 1.0, alveolar PO_2 decreases by about 40–60 mm Hg. If no other problem exists, pulmonary end capillary and systemic arterial PO_2 also decrease by 40 mm Hg.

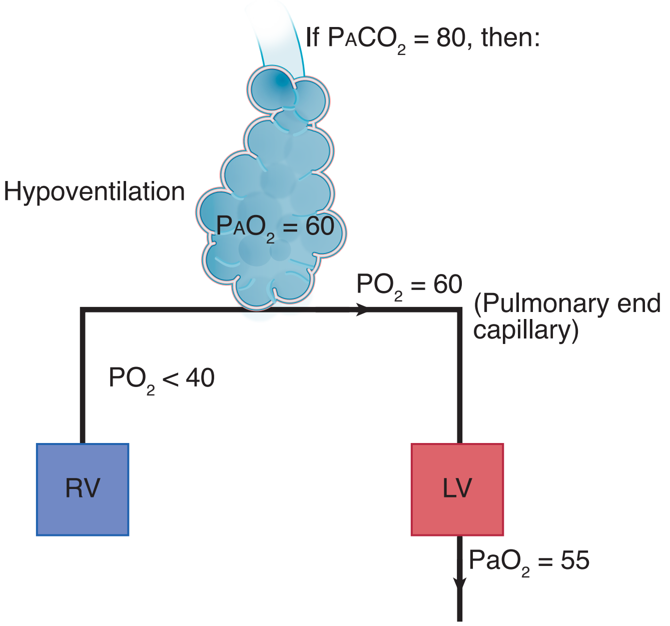


Figure V-4-6. Hypoventilation

Hypoventilation is characterized as an equal decrease in PO_2 in all 3 compartments. **As a result, A–a is normal and end-tidal PO_2 is still a good index of systemic arterial PO_2** (provided A–a gradient is taken into consideration).

The hypoxemia can be relieved by increasing the inspired oxygen, however CO_2 remains elevated because ventilation is unchanged.

In summary:

- There is no increase in the A–a oxygen gradient.
- Supplemental oxygen can relieve the hypoxemia.
- End-tidal air still reflects the systemic arterial compartment.
- The problem is not within the lung itself.

Diffusion Impairment

Diffusion impairment means a structural problem in the lung. As described earlier in this book, this can be produced by a decreased surface area and/or increased thickness of lung membranes.

Clinical Correlate

High altitude is sometimes categorized as a fifth cause of hypoxemia.

High altitude causes low PAO_2, similar to hypoventilation. All the observations described here apply, except for PCO_2. At high altitude, a subject hyperventilates, and thus $PACO_2$ and $PACO_2$ are reduced.

Bridge to Pathology

Acutely, hypoventilation can be caused by narcotics and general anesthetics. More chronic conditions include COPD, kyphoscoliosis, and neuromuscular disorders such as Guillain-Barré, Lambert-Eaton, and myasthenia gravis.

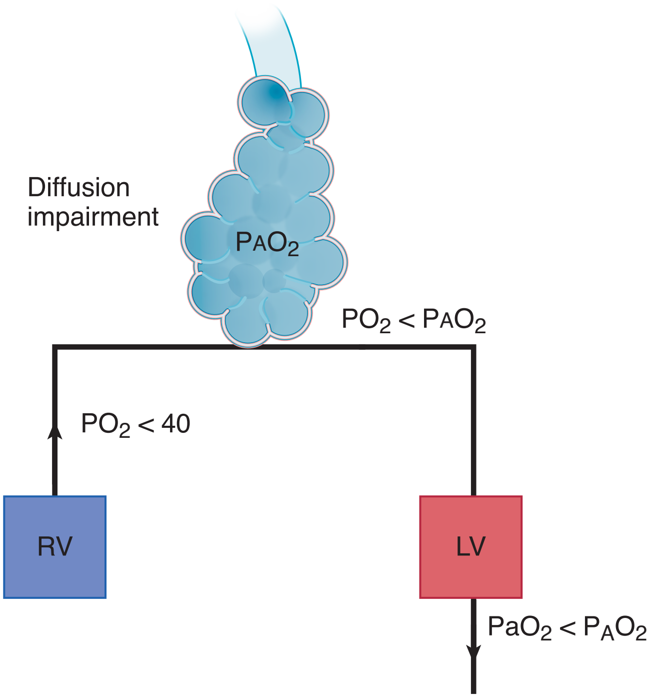


Figure V-4-7. Diffusion Impairment

In marked diffusion impairment, pulmonary end capillary PO_2 is less than alveolar PO_2. End-tidal PO_2 is not a good index of systemic arterial PO_2.

In diffusion impairment, supplemental oxygen corrects the hypoxemia. Note that although the arterial PO_2 may be restored to normal, or even be above normal by supplemental oxygen, there is still an abnormally large A–a gradient.

In summary:

- There is an increase in A–a oxygen gradient.
- Supplemental oxygen can relieve the hypoxemia.
- End-tidal air does not reflect the arterial values.
- It is characterized by a decrease in DLCO.

Bridge to Pathology

Diffusion problems often occur in restrictive pulmonary diseases, such as pulmonary fibrosis, asbestosis, and sarcoidosis. In addition, pulmonary edema can cause a diffusion impairment.

Bridge to Pathology

Some conditions that often result in significant $\dot{V}A/Q$ mismatch include: severe obstruction (status asthmaticus, cystic fibrosis, anaphylaxis), infection (pneumonia), and partial occlusion of an airway (mucus plug, foreign object).

Ventilation-Perfusion Mismatch: Low $\dot{V}A/Q$ Units

If ventilation to a significant portion of the lungs is markedly compromised, then $\dot{V}A/Q$ is << 1.0. As described earlier, low $\dot{V}A/Q$ creates alveolar and end-pulmonary capillary blood gases that are approaching venous gases (low PO_2, and high CO2). The blood from these low $\dot{V}A/Q$ units mixes in with blood draining normal alveolar-capillary units, resulting in systemic hypoxemia.

Because PAO_2 is normal in areas that don't have low $\dot{V}A/Q$, the A–a gradient is elevated. Supplemental oxygen corrects the hypoxemia because the problem regions still have some ventilation—it is just much lower than normal. Similar to diffusion impairment described above, the increased A–a gradient means end-tidal PO_2 is not reflective of PAO_2.

In summary:

- There is an increased A–a oxygen gradient.
- Supplemental oxygen corrects the hypoxemia.
- End-tidal air does not reflect the arterial values.

Intrapulmonary Shunt

By definition, systemic venous blood is delivered to the left side of the heart without exchanging oxygen and carbon dioxide with the alveoli. A right-to-left shunt leads to hypoxemia.

The figure below illustrates the consequences of an intrapulmonary shunt. The solid-line regions represent the normal areas of the lung. The dashed line represents the shunted blood, which is passing from the right heart to the left heart without a change in chemical composition.

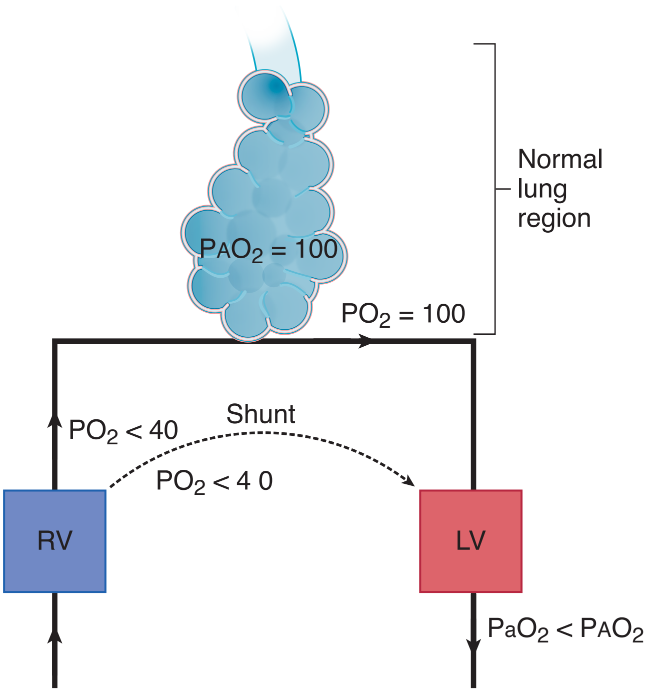


Figure V-4-8. Pulmonary Shunt

With an intrapulmonary shunt, systemic arterial PO_2 is less than alveolar, resulting in an elevated A–a gradient. End-tidal PO_2 does not reflect systemic arterial PO_2.

When a significant intrapulmonary shunt exists, breathing pure O_2 elevates systemic arterial PO_2 a small amount, but it often doesn't correct the hypoxemia. See Figure V-4-9 for response of PAO_2 with shunt.

The failure to obtain a significant increase in arterial PO_2 following the administration of supplemental oxygen in hypoxemia is strong evidence of the presence of a shunt.

Bridge to Pathology

Intrapulmonary shunts are caused by atelectatic lung regions (pneumothorax, ARDS), complete occlusion of an airway (mucus plug, foreign body), and the right-to-left shunts created by heart defects, tetralogy of Fallot, for example.

In summary:

- Increase in A–a oxygen gradient
- Supplemental oxygen ineffective at returning arterial PO_2 to normal
- End-tidal air does not reflect the arterial values

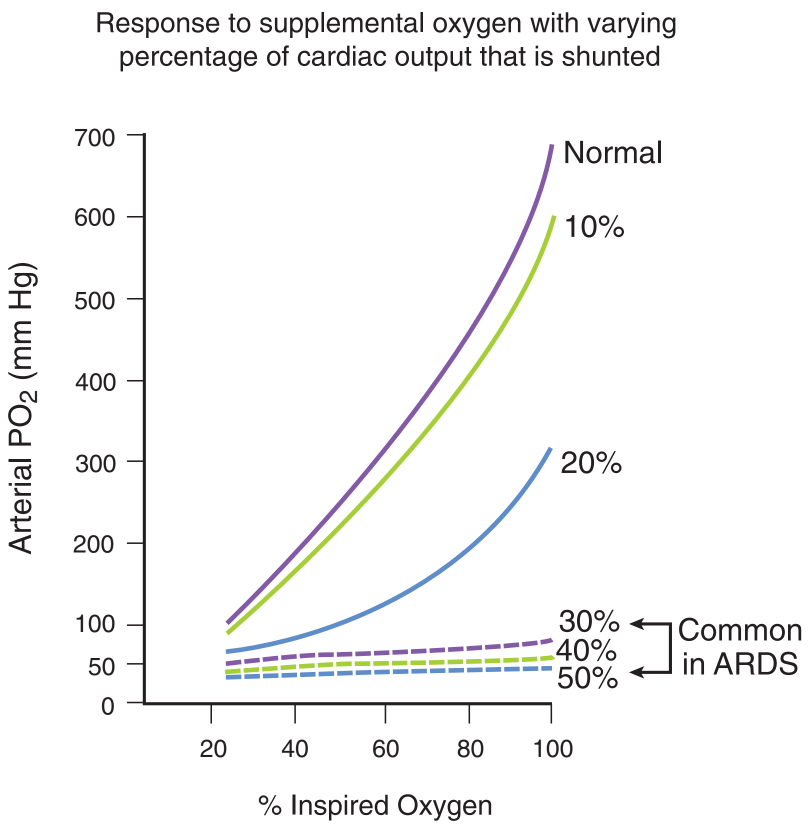


Figure V-4-9. Response to Supplemental Oxygen

LEFT-TO-RIGHT SHUNTS

Pressures are usually higher on the left side of the heart (atria and ventricles), and thus flow is normally left to right. A major characteristic is that hypoxemia never develops in a left-to-right shunt. The principal example is an atrial or ventricular septal defect.

The normal PO_2 values in the left and right compartments can be seen below. Note from the descriptions that follow where the first increase in PO_2 develops on the right side.

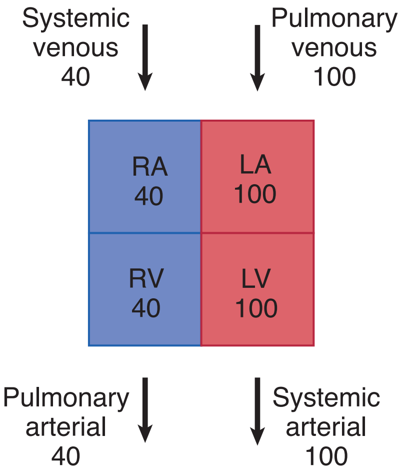


Figure V-4-10. Left-to-Right Cardiac Shunts

Numbers refer to normal PO_2 in mm Hg

- Diagnosed clinically with echocardiogram with bubble study
- Most intracardiac shunts are left-to-right shunts. However, longstanding uncorrected shunts result in a reversal of the shunt.

Table V-4-1. Consequences of 3 Left-to-Right Shunts

	Atrial Septal Defect	Ventricular Septal Defect	Patent Ductus (newborn)
Systemic arterial PO_2	No change	No change	No change
Right atrial PO_2	↑	No change	No change
Right ventricular PO_2	↑	↑	No change
Pulmonary arterial PO_2	↑	↑	↑
Pulmonary blood flow	↑	↑	↑
Pulmonary arterial pressure	↑	↑	↑

Atrial septal defect: PO_2 increase first appears in right atrium

Ventricular septal defect: PO_2 increase first appears in right ventricle

Patent ductus: PO_2 increase appears in pulmonary artery

Recall Question

In which of the following ways does myasthenia gravis cause hypoxemia?

A. Neuromuscular junction pathology causes hypoventilation, leading to chronic hypoxemia

B. Increases the A-a oxygen gradient

C. Fibrosis and sclerosis of the alveoli cause diffusion impairment

D. Ventilation-perfusion mismatch caused by a fibrotic scar form in the apex of the lung

E. Complete occlusion of an airway caused by a sclerotic foreign body

Answer: A

PART VI

Renal Physiology

Renal Structure and Glomerular Filtration 1

Learning Objectives

- ❑ Use knowledge of overview of the renal system
- ❑ Demonstrate understanding of nephron hemodynamics
- ❑ Demonstrate understanding of glomerular filtration

THE RENAL SYSTEM

Functions of the Kidney

- Excrete waste products: urea, uric acid, creatinine
- Water and electrolyte balance
- Acid/base balance
- Secrete the hormone erythropoietin and the enzyme renin into the circulation
- Hydroxylate 25-hydroxy-Vit D to form the active form of vitamin D (1,25 dihydroxy-Vit D)

Functional Organization of the Kidney

The figure below illustrates the cortical versus the medullary organization of the kidney. Nephrons (the functioning unit of the kidney) with glomeruli in the outer cortex have short loops of Henle (cortical nephrons). Those with glomeruli in the inner cortex have long loops of Henle that penetrate the medullary region (juxtamedullary nephrons).

- 7/8 of all nephrons are cortical nephrons
- 1/8 of all nephrons are juxtamedullary nephrons

Nephron structures in the medulla consist of the long loops of Henle and the terminal regions of the collecting ducts. All other structures, including the first section of the collecting ducts, are in the cortex.

- In the cortex, the proximal and distal tubules, as well as the initial segment of the collecting duct, are surrounded by a capillary network, and the interstitium is close to an isotonic environment (300 mOsm/kg).
- The medullary region has capillary loops organized similar to the loops of Henle, known as the vasa recta.
- The slow flow through these capillary loops preserves the osmolar gradient of the interstitium.

- However, this slow flow also keeps the PO_2 of the medulla lower than that in the cortex and even though the metabolic rate of the medulla is lower than in the cortex, it is more susceptible to ischemic damage.

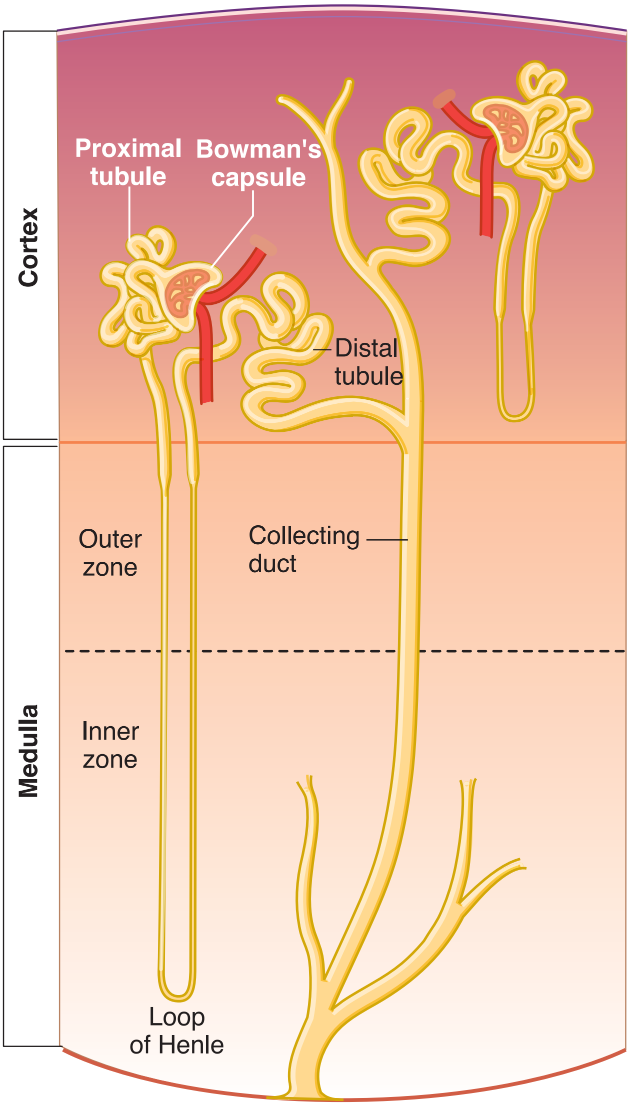


Figure VI-1-1. Nephron Structures

Function of the Nephron

There are 4 basic renal processes: filtration, reabsorption, secretion, and excretion.

Filtration

- Blood is filtered by nephrons, the functional units of the kidney.
- Each nephron begins in a renal corpuscle (site of filtration), which is composed of a glomerulus enclosed in a Bowman's capsule.
- An ultrafiltrate resembling plasma enters Bowman's space.
- Filtration is driven by Starling forces.
- The ultrafiltrate is passed through, in turn, the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and a series of collecting ducts to form urine.
- Filtration rate or filtered load is the amount of a substance (in mg) that is filtered at the glomeruli in a min (mg/min; *see* chapter 2 for more details).

Reabsorption

- Tubular reabsorption is the process by which solutes and water are removed from the tubular fluid that was formed in Bowman's space and transported into the blood.
- Reabsorption rate is the amount (in mg) that is reabsorbed from the ultrafiltrate in a min (mg/min; *see* chapter 2 for more details).

Secretion

- Tubular secretion is the transfer of materials from peritubular capillaries to the renal tubular lumen.
- Tubular secretion is primarily the result of active transport.
- Usually only a few substances are secreted.
- Many drugs are eliminated by tubular secretion.
- Secretion rate is the amount (in mg) that is secreted into the ultrafiltrate in a min (mg/min; *see* chapter 2 for more details).

Excretion

- Substances that are in the urine are excreted.
- A substance that is filtered and not completely reabsorbed is excreted in the urine.
- A substance that is filtered and then secreted is excreted in large amounts in the urine because it comes from 2 places in the nephron.
- Excretion rate is the amount (in mg) that is excreted in the urine in a min (mg/min; *see* chapter 2 for more details).

Clinical Correlate

Spinal cord injury can markedly alter the micturition reflex. A lumbar lesion can eliminate voluntary control (motor nerves exit L1–L3) and the sympathetic component (T10–T12). Over time, the PNS component can return (S1–S3) and voiding can be initiated when the bladder is sufficiently filled.

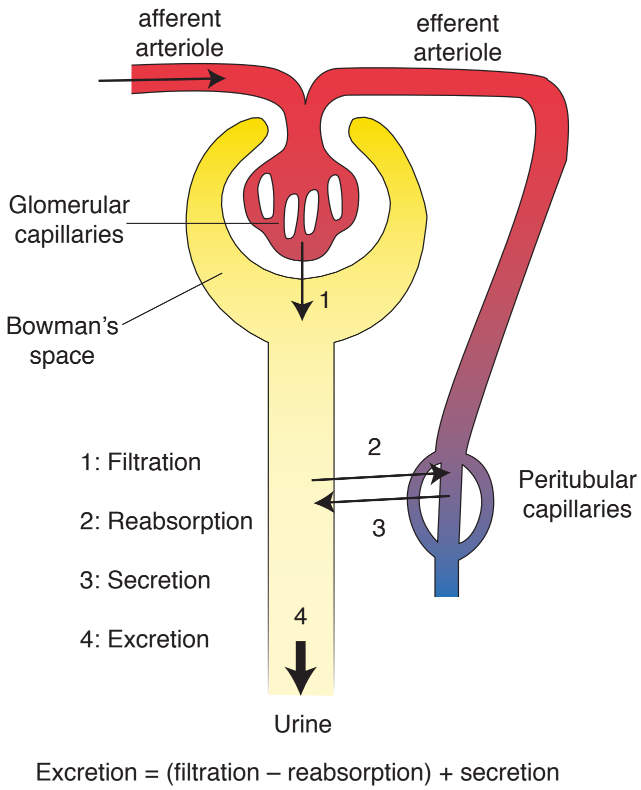


Figure VI-1-2. Renal Processes

The following equation is central to understanding renal physiology and will be addressed in detail in a later chapter.

$$\text{Excretion rate (ER)} = (\text{filtration rate} - \text{reabsorption rate}) + \text{secretion rate}$$

Micturition Reflex

Micturition is a reflex regulated by the peripheral nervous system. The autonomic component exists at birth, continuing throughout life, but the motor component requires sufficient maturation of the nervous system (occurs around age 2). This section discusses the physiologic regulation that occurs in sufficiently mature individuals. In this case, nuclei in the medulla ultimately regulate the phase, switching between the filling and voiding phases.

Filling phase

- This phase is typically the longest and is dominated by the sympathetic nervous system (SNS).
- Sympathetic input relaxes the detrusor muscle via the β-3 receptor (Gs-cAMP). In addition, sympathetic input contracts the internal sphincter via α-1 receptors.
- As a result, the bladder can fill with urine.

Voiding phase

- As the bladder fills, the pressure of the fluid causes distension of the bladder.
- This distension activates sensory afferent neurons (not depicted in figure) resulting in activation of the parasympathetic nervous system (PNS) and inhibition of the SNS (spinal reflex). In addition, in the sufficiently mature individual, it sends input to the medulla and cortex signaling that voiding is needed.
- PNS activation causes contraction of the detrusor muscle (M3). This initiates voiding.
- However, the external sphincter is controlled voluntarily (nicotinic receptor). If voiding is inappropriate at that moment, voluntary contraction of this sphincter stops the voiding process.
- If the voiding reflex is thwarted voluntarily, the bladder initially relaxes (stretch-induced relaxation of smooth muscle), reducing the pressure and the sensory drive to void.
- However, continued filling of the bladder increases pressure and re-initiates the sensory input attempting to start the voiding process.
- Typically, one voluntarily relaxes the external sphincter by inhibiting motor output (Ach via nicotinic receptor) and the bladder is emptied.

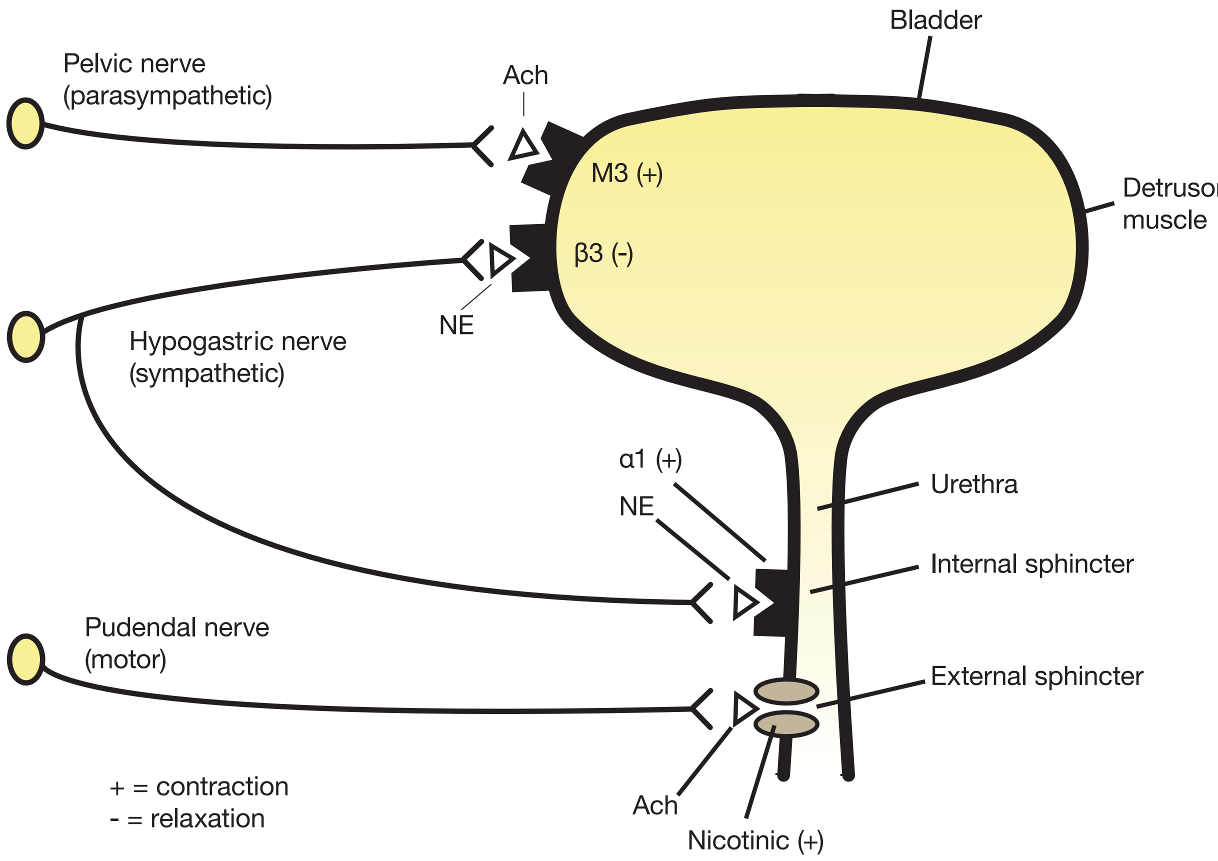


Figure VI-1-3. Control of Micturition

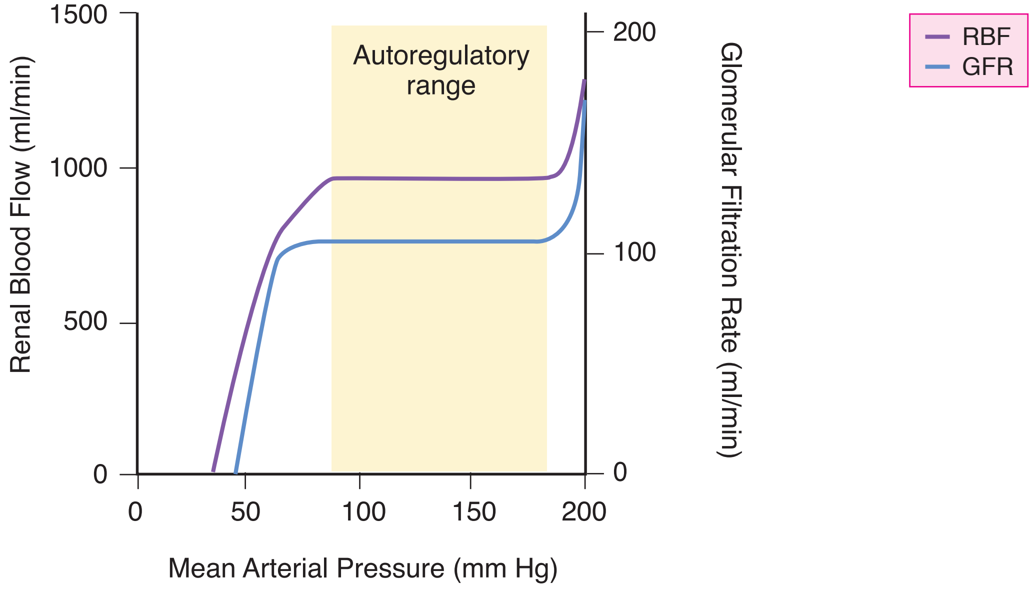


Figure VI-1-4. Autoregulation and the Renal Function Curve

NEPHRON HEMODYNAMICS

Autoregulation

Blood flow throughout the kidney: renal artery → arcuate artery → afferent arteriole → glomerular capillaries → efferent arterioles → peritubular capillaries → vasa recta → arcuate vein → renal vein

The kidneys are very effective in autoregulating blood flow. This is primarily due to changes in the resistance of the afferent arterioles, for which 2 mechanisms are involved:

- **Myogenic responses**: the intrinsic property of smooth muscle is to contract when stretched (*see* CV chapter)
- **Tubuloglomerular feedback (TGF)**
  - Increased MAP leads to an increase in RBF and GFR
  - High delivery of sodium ions to the macula densa (the part of the nephron where the thick ascending loop of Henle connects with the beginning of the distal tubule) → adenosine and ATP secretion → vasoconstriction of the afferent arteriole → decreases renal blood flow and GFR.
  - Decreased delivery of sodium to the macula densa dilates the arteriole and leads to an increase in renal blood flow and GFR

Series Hemodynamics

The individual nephrons that make up both kidneys are connected in parallel. However, the flow through a single nephron represents 2 arterioles and 2 capillary beds connected in series.

Flow must be equal at all points in any series system. If flow changes, it changes equally at all points in the system.

Flow (Q) = pressure gradient / resistance (R) = (upstream pressure-downstream pressure)

Blood flows from high pressure to low pressure. Two factors decrease flow:

- Decreasing the pressure gradient (decreasing the upstream pressure or increasing the downstream pressure)
- Increasing resistance at any point throughout the circuit

Therefore, when considering blood flow through the nephron as a series circuit, if resistance increases (vasoconstriction) at the afferent arteriole or efferent arteriole, renal plasma flow decreases.

When an arteriole vasoconstricts, this increases the resistance at that arteriole and there are 2 changes to consider:

- Flow across the entire circuit **decreases**
- Pressure builds up or **increases** before (upstream) the point of resistance **and** pressure **decreases** after (downstream) the point of resistance

When an arteriole vasodilates, this decreases the resistance at that arteriole, and there are 2 changes to consider:

- Flow across the entire circuit **increases**
- Pressure **decreases** before (upstream) the point of resistance **and** pressure **rises** after (downstream) the point of resistance

Hemodynamics of a single nephron

The hemodynamics of a single nephron can be seen below. Connected in series are the high-pressure filtering capillaries of the glomerulus and the low-pressure reabsorbing peritubular capillaries.

The glomerular capillaries have a very high hydrostatic pressure because the efferent arterioles are very narrow and thus have a very high resistance. Likewise, there is a large pressure drop as blood flows past this high resistant arteriole and the peritubular capillaries have very low hydrostatic pressure.

R$_1$: afferent arteriole

R$_2$: efferent arteriole

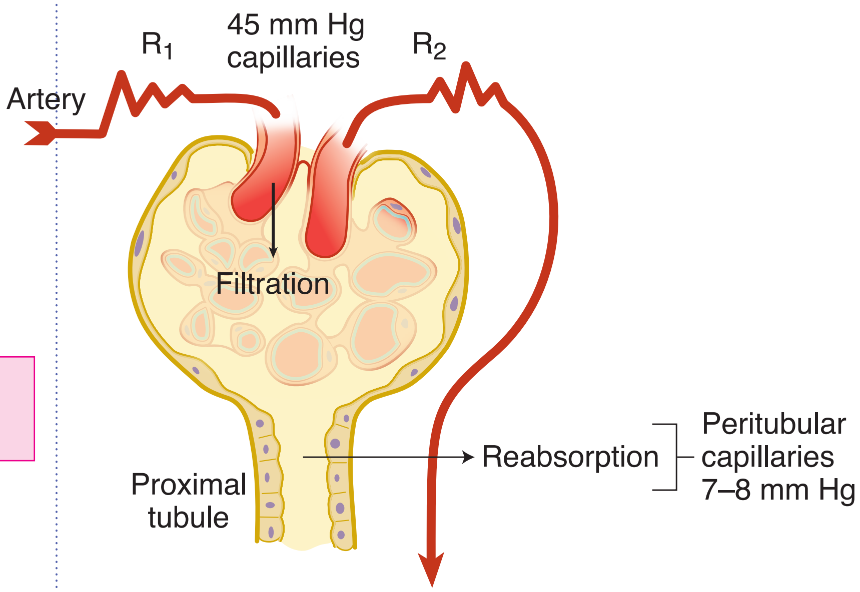


Figure VI-1-5. Glomerular Hemodynamics

Independent response of the afferent and efferent arterioles

The table below illustrates the expected consequences of independent isolated constrictions or dilations of the afferent and efferent arterioles.

Table VI-1-1. Consequences of Independent Isolated Constrictions or Dilations of the Afferent and Efferent Arterioles

	Glomerular Cap Pressure	Peritubular Cap Pressure	Nephron Plasma Flow
Constrict efferent	↑	↓	↓
Dilate efferent	↓	↑	↑
Constrict afferent	↓	↓	↓
Dilate afferent	↑	↑	↑

GLOMERULAR FILTRATION

Glomerular filtration rate (GFR) is the rate at which fluid is filtered into Bowman's capsule. The units of filtration are volume filtered per unit time, e.g., mL/min or liters/day; in a young healthy adult it is about 120 mL/min or 180 L/day.

If one kidney is removed (half of the functioning nephrons lost), GFR decreases only about 25% because the other nephrons compensate.

Factors Determining Net Filtration Pressure

There are 4 factors that determine net filtration pressure.

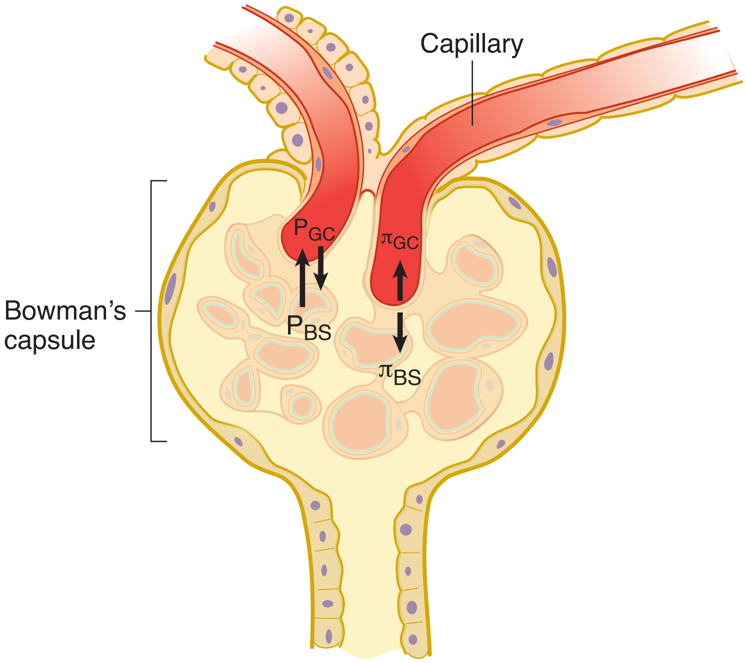


Figure VI-1-6. Determinants of Filtration

P_{GC}: hydrostatic pressure of glomerular capillary

π_{GC}: oncotic pressure of glomerular capillary

P_{BS}: hydrostatic pressure of Bowman's space

π_{BS}: oncotic pressure of Bowman's space

Hydrostatic pressure of the glomerular capillaries

PGC: The hydrostatic pressure of the glomerular capillaries is the only force that promotes filtration. Under normal conditions, this is the main factor that determines GFR.

Oncotic pressure of the plasma

πGC: The oncotic pressure of the plasma varies with the concentration of plasma proteins. Because fluid is filtered but not protein, oncotic pressure, which opposes filtration, increases from the beginning to the end of the glomerular capillaries.

Hydrostatic pressure in Bowman's space

PBS: The hydrostatic pressure in Bowman's capsule opposes filtration. Normally, it is low and fairly constant and does not affect the rate of filtration. However, it increases and reduces filtration whenever there is an obstruction downstream, such as a blocked ureter or urethra (postrenal failure).

Protein or oncotic pressure in Bowman's space

πBS: This represents the protein or oncotic pressure in Bowman's space. Very little if any protein is present, and for all practical purposes this factor can be considered zero.

Normal Values

PBS = 8 mm Hg

PGC = 45 mm Hg

πBS = 0 mm Hg

πGC = 24 mm Hg

Net filtration pressure = PGC – πGC – PBS = 45 – 24 – 8 = 13 mm Hg

Bridge to Pathology

In **nephrotic syndrome** there is marked disruption of the filtering membrane. As a result, plasma proteins now pass through the membrane and are eliminated in the urine. This is typically associated with a non-inflammatory injury to the glomerular membrane system.

Most common clinical signs:

- Marked proteinuria >3.5 gm/day (because of disrupted glomerular membrane system)
- Edema (loss of plasma oncotic pressure)
- Hypoalbuminemia (albumin lost in urine)
- Lipiduria (disrupted membrane system and proteins in urine)
- Hyperlipidemia (increased lipid synthesis in liver)

In nephritic syndrome, there is an inflammatory disruption of the glomerular membrane system. This disruption allows proteins and cells to cross the filtering membrane. The most common clinical signs are:

- Proteinuria <3.5 gm/day (evidence of disrupted membrane)
- Hematuria (disrupted membrane)
- Oliguria (inflammatory infiltrates reduce fluid movement across the membrane)
- Hypertension (inability of kidney to regulate the extracellular volume)
- Azotemia (inability to filter and excrete urea)

To summarize, filtration at the glomeruli depends on Starling forces:

- The glomerular capillaries have very high hydrostatic pressures (this is why filtration occurs here)
- Increasing the glomerular hydrostatic pressure → increases GFR
- Decreasing the glomerular hydrostatic pressure → decreases GFR

Oncotic pressure opposes GFR → ↑ plasma protein → ↑ oncotic pressure → ↓ GFR (no effect on RPF) ↓ plasma protein → ↓ oncotic pressure → ↑ GFR (no effect on RPF)

The increased concentration of protein (increased oncotic pressure) is carried into the peritubular capillaries and promotes a greater net force of reabsorption.

Important: If the main driving force for GFR is the hydrostatic pressure, what is the main driving force for the reabsorption at the proximal tubule? The force that is driving reabsorption at the proximal tubule is the oncotic pressure in the peritubular capillaries.

Filtering Membrane

The membrane of the glomerulus consists of 3 main structures:

- Capillary endothelial wall with fenestrations that have a magnitude greater than proteins; in addition, the wall is covered with negatively charged compounds
- Glomerular basement membrane made up of a matrix of extracellular negatively charged proteins and other compounds
- Epithelial cell layer of podocytes next to Bowman's space; the podocytes have foot processes bridged by filtration slit diaphragms

Around the capillaries is the mesangium, containing mesangial cells similar to monocytes.

The capillary wall with its fenestrated endothelium, the basement membrane with hydrated spaces, and the interdigitating foot processes of the podocytes combined with an overall large surface area, create a high hydraulic conductivity (permeable to water and dissolved solutes). Passage of large proteins is restricted because of negative charge of the membrane system.

In addition to the net hydraulic force, GFR depends on both the permeability and the surface area of the filtering membrane. The decrease in GFR in most diseased states is due to a reduction in the membrane surface area. This also includes a decrease in the number of functioning nephrons.

Materials Filtered

The following are **easily or freely filtered**:

- Major electrolytes: sodium, chloride, potassium, bicarbonate
- Metabolic waste products: urea, creatinine
- Metabolites: glucose, amino acids, organic acids (ketone bodies)
- Nonnatural substances: inulin, PAH (p-aminohippuric acid)
- Lower-weight proteins and peptides: insulin, myoglobin

The following are **not freely filtered**:

- Albumin and other plasma proteins
- Lipid-soluble substances transported in the plasma attached to proteins, such as lipid-soluble bilirubin, T4 (thyroxine), other lipid-soluble hormones; unbound lipid-soluble substances such as free-cortisol are filtered and can appear in the urine

As blood flows though the glomerular capillary, plasma is filtered, but albumin is not, so the plasma albumin concentration and oncotic pressure increase.

Fluid Entering Bowman's Capsule

The fluid entering Bowman's space is an ultrafiltrate of plasma; that is, the filtrate has the same concentration of dissolved substances as plasma, except proteins. The osmolality of the filtrate is 300 mOsm/kg. The criteria for effective osmolality are the same as those previously stated for extracellular fluid (part I).

If a substance is freely filtered by the kidney, the ratio of the filtrate concentration to plasma concentration TF/P = 1.0. This means the concentrations in Bowman's space and the plasma are the same.

Filtration Fraction

The following formula for filtration fraction (FF) and the normal values given should be memorized.

FF = fraction of the material entering the kidney that is filtered
normally 0.20 or 20% for a freely filtered substance

$$FF = \frac{GFR}{RPF} \qquad \begin{array}{l} GFR = 120\ mL/min \\ RPF\ (renal\ plasma\ flow) = 600\ mL/min \end{array}$$

$$= \frac{120\ mL/min}{600\ mL/min} = 0.20 \text{ or } 20\%$$

FF affects oncotic pressure in the peritubular capillary (πPC). The greater the FF, the higher the oncotic pressure in the peritubular capillaries; that is because FF represents loss of protein-free fluid into Bowman's space, thereby increasing the concentration of protein in the plasma.

- If FF decreases, then πPC decreases
- Only 20% of the renal plasma flow is filtered. Every minute 600 ml of plasma enters the kidneys. That is the renal plasma flow.
- 20% or 120 mL of plasma is filtered hence a GFR of 120 mL.

Recall Question

Which of the following best indicates the effect of a drug that dilates the efferent arterioles of the kidney?

A. Decreased glomerular cap pressure, decreased peritubular cap pressure, increased nephron plasma flow

B. Decreased glomerular cap pressure, increased peritubular cap pressure, increased nephron plasma flow

C. Decreased glomerular cap pressure, decreased in peritubular cap pressure, decreased in nephron plasma flow

D. Increased glomerular cap pressure, increased in peritubular cap pressure, increased in nephron plasma flow

E. Increased glomerular cap pressure, decreased peritubular cap pressure, increased nephron flow

Answer: B

Factors Affecting FF

Based on the preceding discussion, the following should be expected for afferent versus efferent constriction:

	Afferent Constriction	Efferent Constriction
Glomerular filtration pressure	↓	↑
GFR	↓	↑
RPF	↓	↓
FF	↔	↑

Effects of Sympathetic Nervous System

Stimulation of the sympathetic neurons to the kidney causes vasoconstriction of the arterioles, but has a greater effect on the afferent arteriole. As a consequence:

- RPF decreases
- PGC decreases
- GFR decreases
- FF increases

- PPC decreases
- πPC increases
- Increased forces promoting reabsorption in the peritubular capillaries because of a lower peritubular capillary hydrostatic pressure and an increase in plasma oncotic pressure (FF increases)

Effects of Angiotensin II

Angiotensin II (Ang II) is a vasoconstrictor. It constricts both the afferent and efferent arterioles, but is has a bigger effect on the efferent arteriole. As a consequence:

- RPF decreases
- PGC increases
- GFR increases
- FF increases
- PPC decreases
- πPC increases
- Increased forces promoting reabsorption in the peritubular capillaries because of a lower peritubular capillary hydrostatic pressure and an increase in plasma oncotic pressure (FF increases)

During a stress response, there is an increase in sympathetic input and very high levels of circulating angiotensin II. As a consequence:

- Increased sympathetic tone to the kidneys and very high levels of angiotensin II vasoconstrict both the afferent and the efferent arterioles. Because both arterioles constrict, there is a large drop in the RPF and only a small drop in the GFR.
- The net effect is an increase in FF.
- The increase in FF → increase in oncotic pressure → increase in the reabsorption in proximal tubules
- Overall, less fluid is filtered and a greater percentage of that fluid is reabsorbed in the proximal tubule, leading to preservation of volume in a volume depleted state
- There is also an increase in ADH due to the low volume state
- Activation of the sympathetic nervous system also directly increases renin release

The net effect of angiotensin II is to preserve GFR in volume-depleted state. In a volume-depleted state, a decrease in GFR is beneficial because less fluid filtered results in less fluid excretion (however, a very large decrease in GFR prevents removal of waste products like creatinine and urea). Angiotensin II prevents a large decrease in GFR.

Clinical Correlate

A 25-year-old man spends a week in the desert. Due to severe dehydration, his volume status is depleted → high angiotensin II → vasoconstriction of the efferent arterioles → an increase in GFR and decrease in RPF → an increase in FF → more plasma filtered in the glomeruli → higher albumin concentration (hence higher oncotic pressure) in the glomerular capillaries → higher oncotic pressure in the peritubular capillaries → an increase in peritubular reabsorption. Therefore, an increase in the FF → an increase in reabsorption at the proximal tubules.

A dehydrated patient needs to increase reabsorption of fluid at the proximal tubules to preserve volume. Angiotensin II helps preserve GRF and volume in a volume-depleted state.

Clinical Correlate

What would happen if you gave NSAIDs to the 75-year-old man who is hemorrhaging?

During a stress state the increase in sympathetic tone causes vasoconstriction of the afferent arterioles. The same stimuli activate a local production of prostaglandins. Prostaglandins lead to vasodilation of the afferent arterioles, thus modulating the vasoconstriction. Unopposed, the vasoconstriction from the sympathetic nervous system and angiotensin II can lead to a profound reduction in RPF and GFR, which in turn, could cause renal failure. NSAIDs inhibit synthesis of prostaglandins and interfere with these protective effects.

Clinical Correlate

ACE inhibitors and **ARBs** are used for diabetic nephropathy because they lead to a reduction in glomerular capillary pressure and reduce damage and fibrosis of the glomeruli (which will delay the need for hemodialysis). They treat hyperfiltration. In most cases, there is a small and transient drop in GFR.

Inhibition of angiotensin II leads to vasodilation of the efferent arteriole, which leads to decreased glomerular capillary pressure and decreased GFR. It also leads to increased RPF because of the decrease in resistance to flow. The pressure downstream from the efferent arteriole (peritubular capillary pressure) increases because there is a decreased resistance at the EA.

Use the following guidelines for using ACE inhibitors and ARBs:

- Give ACE inhibitors to patients with nephrotic syndrome and stable chronic renal failure.
- Avoid ACE inhibitors and ARBs in patients with severely compromised GFR (risk of hyperkalemia) and with acute renal failure.
- ACE inhibitors and ARBs may cause a type IV RTA because they block aldosterone (leading to hyperkalemia); in this case they must both be held. If ACE inhibitors cause hyperkalemia, so will ARBs.
- Switch from ACE inhibitor to ARB in cases with ACE-inhibitor cough, not for hyperkalemia.
- ACE inhibitors and ARBs are contraindicated in bilateral renal artery stenosis, where both kidneys have such low perfusion that GFR is highly dependent on constriction of EA. When the effect of angiotensin II is removed, the result is a significant drop in GFR and acute renal failure.

There is no parasympathetic innervation of the kidney.

Although prostaglandins seem to play little role in the normal regulation of renal blood flow, they do become important in times of stress. For example, the vasoconstriction produced by sympathetic activation is partially countered by the local release of vasodilating prostaglandins (PGI_2 and PGE_2). This is thought to help prevent ischemic damage during times of stress.

Solute Transport: Reabsorption and Secretion

2

Learning Objectives

- ❑ Interpret scenarios on solute transport
- ❑ Interpret scenarios on quantifying renal processes (mass balance)
- ❑ Demonstrate understanding of clearance
- ❑ Answer questions about TM tubular reabsorption
- ❑ Solve problems concerning TM tubular secretion
- ❑ Use knowledge of the renal handling of some important solutes

SOLUTE TRANSPORT

Transport proteins in the cell membranes of the nephron mediate the reabsorption and secretion of solutes and water transport in the kidneys. Acquired defects in transport proteins are the cause of many kidney diseases.

In addition the transport proteins are important drug targets.

Transport Mechanisms

Simple diffusion

- Net movement represents molecules or ions moving down their electrochemical gradient.
- This doesn't require energy.

Facilitated diffusion (facilitated transport)

- A molecule or ion moving across a membrane down its concentration gradient attached to a specific membrane-bound protein.
- This doesn't require energy.

Active transport

- A protein-mediated transport that uses ATP as a source of energy to move a molecule or ion against its electrochemical gradient.

Dynamics of Protein-Mediated Transport

Uniport

- Transporter moves a single molecule or ion as in the uptake of glucose into skeletal muscle or adipose tissue. This is an example of facilitated diffusion.

Symport (cotransport)

- A coupled protein transport of 2 or more solutes in the same direction as in Na-glucose, Na-amino acid transporters.

Antiport (countertransport)

- A coupled protein transport of 2 or more solutes in the opposite direction.

Generally, protein carriers transport substances that cannot readily diffuse across a membrane. There are no transporters for gases and most lipid-soluble substances because those substances readily move across membranes by simple diffusion.

Characteristics common to all protein-mediated transport

Rate of transport: A substance is transported more rapidly than it would be by diffusion, because the membrane is not usually permeable to any substance for which there is a transport protein.

Saturation kinetics: As the concentration of the substance initially increases on one side of the membrane, the transport rate increases.

- Once the transporters become saturated, transport rate is maximal (TM = transport maximum). Rate of transport is dependent upon:
  - Concentration of solute
  - Number of functioning transporters; the only way to increase TM is to add more protein carriers to the membrane
- Once all the protein carriers are saturated, the solutes are transported across the membrane at a constant rate. This constant rate is TM.
- There is no TM in simple diffusion.

Chemical specificity: To be transported, the substance must have a certain chemical structure. Generally, only the natural isomer is transported (e.g., D-glucose but not L-glucose).

Competition for carrier: Substances of similar chemical structure may compete for the same transporter. For example, glucose and galactose generally compete for the same transport protein.

Primary and secondary transport

- In primary active transport, ATP is consumed directly by the transporting protein, (e.g., the Na/K-ATPase pump, or the calcium-dependent ATPase of the sarcoplasmic reticulum).

- Secondary active transport depends indirectly on ATP as a source of energy, as in the cotransport of Na-glucose in the proximal tubule. This process depends on ATP utilized by the Na/K-ATPase pump.
- Glucose moves up a concentration gradient via secondary active transport.

The figure below represents a renal proximal tubular cell.

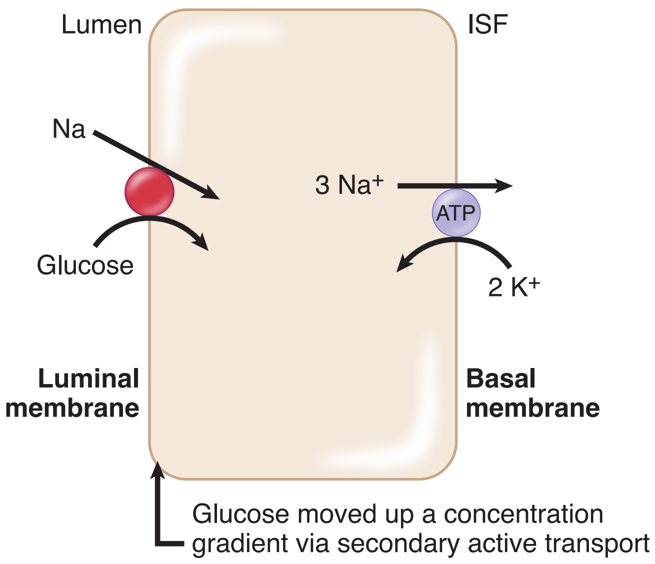


Figure VI-2-1. Renal Tubule or Small Intestine

The Na/K-ATPase pump maintains a low intracellular sodium concentration, which creates a large gradient across the cell membrane. It is this sodium gradient across the luminal membrane that drives secondary active transport of glucose.

In summary, the secondary active transport of glucose:

- Depends upon luminal sodium
- Is stimulated by luminal sodium (via increased sodium gradient)
- Is linked to the uptake of sodium
- Depends upon rate of metabolic ATP production

Another example of secondary active transport is the counter transport of Na–H^+ also in the proximal tubule. This process depends on the Na/K-ATPase pump.

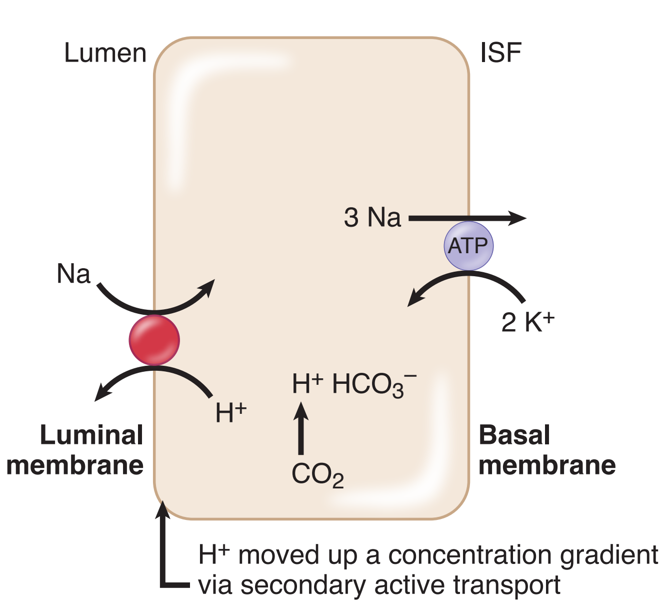


Figure VI-2-2. Proximal Tubule

QUANTIFYING RENAL PROCESSES (MASS BALANCE)

As stated earlier, there are 4 processes in the nephron: filtration, reabsorption, secretion, and excretion. The figure below illustrates that how the nephron handles any solute—on a net basis—can be derived because the rate at which it enters (filtered load) and its rate of excretion can be measured.

Both variables are expressed as an amount of substance per unit time, and the units are the same, e.g., mg/min.

U_X: urine concentration of substance

V: urine flow rate

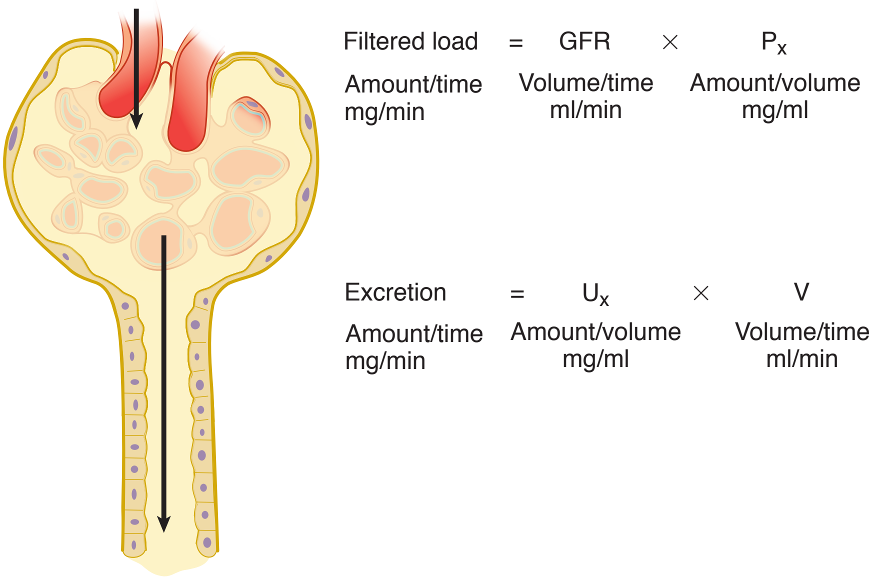


Figure VI-2-3. Relationship of Filtered Load and Excretion

No Net Tubular Modification

- Filtered load = excretion rate
- The amount filtered and amount excreted per unit time are always the same, e.g., inulin, mannitol.

Net Reabsorption

- Filtered load > excretion
- Excretion is always less than filtered load, e.g., glucose sodium, urea.
- If the substance is completely reabsorbed, the rate of filtration and the rate of reabsorption are equal.
- Excretion rate is 0
- If the substance is partially reabsorbed, excretion is less than filtration.

Net Secretion

- Filtered load < excretion
- Excretion is always greater than filtered load, e.g., PAH, creatinine.
- Creatinine is freely filtered, and a very small amount is secreted.

The following formula is sometimes used to calculate net transport. The sign of the calculated number will indicate the 3 basic categories:

0 = no net transport
+ = net reabsorption
– = net secretion

net transport rate = filtered load – excretion rate

$$= (GFR \times Px) - (Ux \times V)$$

Problem: Given the following information, calculate the reabsorption rate for glucose.

GFR = 120 mL/min
Plasma glucose = 300 mg/100 mL
Urine flow = 2 mL/min
Urine glucose = 10 mg/mL

Answer: 340 mg/min

GFR = glomerular filtration rate
units = volume/time, e.g., mL/min

P_x = free (not bound to protein) concentration of substance in plasma
units = amount/volume, e.g., mg/mL

CLEARANCE

Clearance refers to a theoretical volume of plasma from which a substance is removed over a period of time. Applying the principles of mass balance above, if a solute has an ER, then it is cleared by the kidney. In other words, if it is filtered and not fully reabsorbed or is secreted, then it appears in the urine and is thus cleared from the body.

If, on the other hand, it is filtered and then all is reabsorbed, the ER and clearance is zero, and it is not cleared by the kidney.

For example, if the concentration of substance x is 4 molecules per liter and the excretion of x is 4 molecules per minute, the volume of plasma cleared of x is 1 L per minute.

If the excretion of x decreases to 2 molecules per minute, the volume cleared of x is only 0.5 L per minute. If the concentration of x decreases to 2 molecules per liter of plasma and excretion is maintained at 2 molecules per minute, the cleared volume is back to 1 L per minute.

Table VI-2-1. Example Calculations of Clearance Values

Plasma Concentration (molecules/L)	Excretion Rate (molecules/minute)	Volume Cleared (L/minute)
4	4	1.0
4	2	0.5
2	2	1.0

Ux: urine concentration of x

V: urine flow rate

Px: plasma concentration of x

Thus, the factors which determine clearance are the plasma concentration of the substance and its excretion rate.

$$\text{Clearance of x} = \frac{\text{Excretion rate of x}}{\text{Px}} = \frac{\text{Ux} \times \text{V}}{\text{Px}}$$

The plasma concentration of the substance and its urine concentration must be in the same units, which then cancel.

Urine flow (V) is a volume per unit time, and the units of V become the units of clearance.

Clearance is a volume of plasma cleared of a substance per unit time, mL/min or L/day.

Problem: Using the following information, calculate the clearance of x, y, and z.

V = 2 mL/min

Ux = 2 mg/mL	Px = 2 mg/mL
Uy = 0 mg/mL	Py = 13.6 mg/mL
Uz = 0.5 mg/mL	Pz = 1 mg/mL

Answer: *x* = 2 mL/min, y = 0, and z = 1 mL/min

TM TUBULAR REABSORPTION

Glucose

The dynamics of glucose filtration, reabsorption, and excretion are seen below, applying the mass balance and clearance principles discussed earlier. Many substances are reabsorbed via a TM system, and glucose serves as our prototypical example.

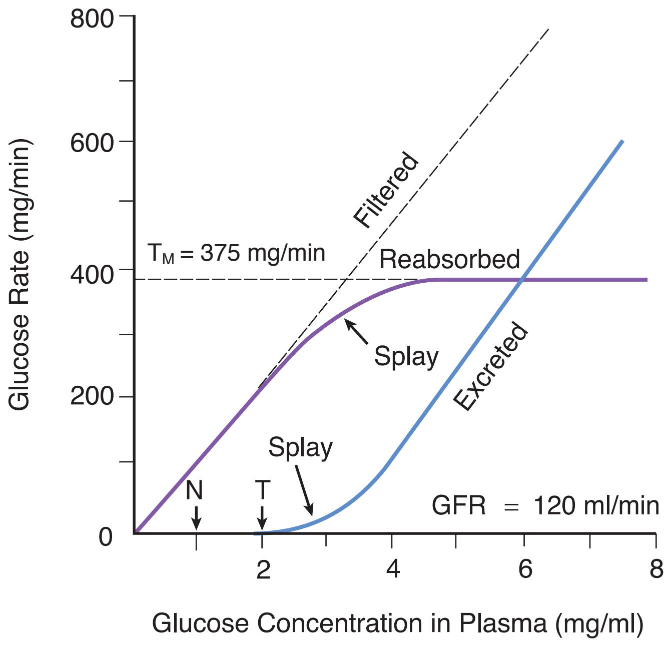


Figure VI-2-4. Transport Maximum Reabsorption of Glucose

N: normal plasma glucose concentration

T: plasma (renal) threshold

The y-axis is glucose rate (mg/min). There are 3 rates: filtration (dashed line), excretion (blue line); and reabsorption (purple line), which is filtered load (filtration rate) – excretion rate (ER).

- At low plasma levels, the filtration and reabsorption rates of glucose are equal, thus glucose does not appear in the urine and the clearance is zero.
- TM is the maximal reabsorption rate of glucose, i.e., the rate when all the carriers (SGLT-2/1) are saturated. TM can be used as an index of the number of functioning nephrons.
- The rounding of the reabsorption curve into the plateau is called **splay**. Splay occurs because some nephrons reach TM before others. Thus, TM for the entire kidney is not reached until after the region of splay.
- Plasma (or renal) threshold is the plasma glucose concentration at which glucose first appears in the urine. This occurs at the beginning of splay. Before splay, all of the glucose that is filtered is reabsorbed and the ER is 0.

TM TUBULAR SECRETION

P-Aminohippuric Acid Secretion

P-aminohippuric acid (PAH) secretion from the peritubular capillaries into the proximal tubule is an example of a transport maximum system. As a TM system, it has the general characteristics discussed for the reabsorption of glucose except for the direction of transport.

The figure below illustrates the renal handling of PAH at low plasma concentrations.

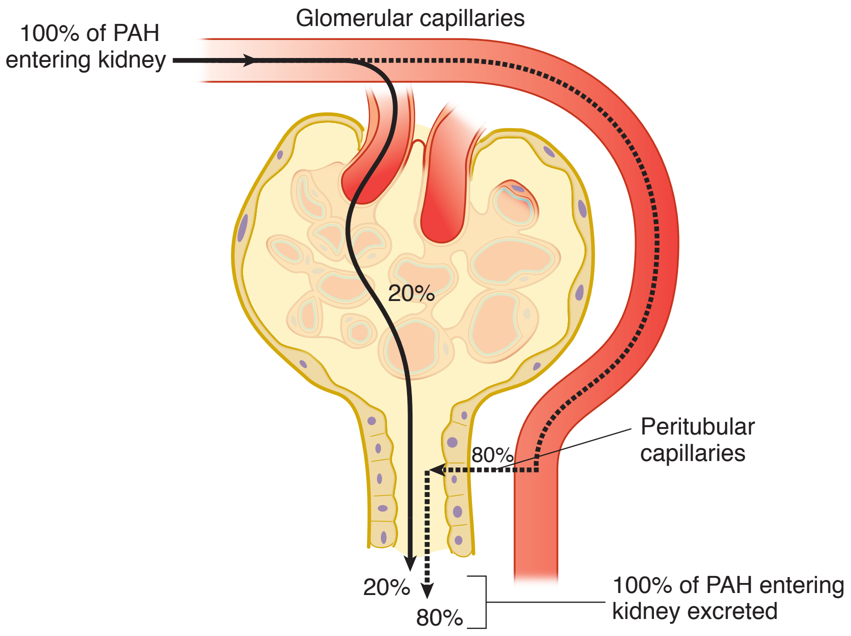


Figure VI-2-5. Secretion of PAH

Normal values:

Renal plasma flow = 600 mL/min

GFR = 120 mL/min

FF = 0.20

- The renal plasma flow (RPF) is the volume of plasma that enters the kidney in a minute (600 mL/min).
- The RPF contains the total concentration of PAH dissolved in plasma in mg/ml entering the PAH.
- Of the RPF, 20% (120 mL) is typically filtered, regardless of the total amount of PAH entering the kidney (in the RPF).
- Whatever is filtered is excreted, it is NOT reabsorbed; therefore, that 20% is **always** cleared (removed from the plasma) and excreted (placed in the urine)!!
- If the plasma concentration is below the TM for PAH, then the remaining 80% in the plasma is secreted into the lumen.
- Since PAH is not reabsorbed, then all the PAH is cleared by the kidney and thus the clearance of PAH provides effective renal plasma flow (ERPF). It is called ERPF because a portion of the blood flow entering the kidney does not go to the glomerulus (perfuses renal capsule).
- Determining ERPF allows one to estimate RBF:

RBF = RPF/1 – hematocrit (HCT)

For example, suppose the concentration of PAH in the plasma entering the kidney is 1 mg/ml.

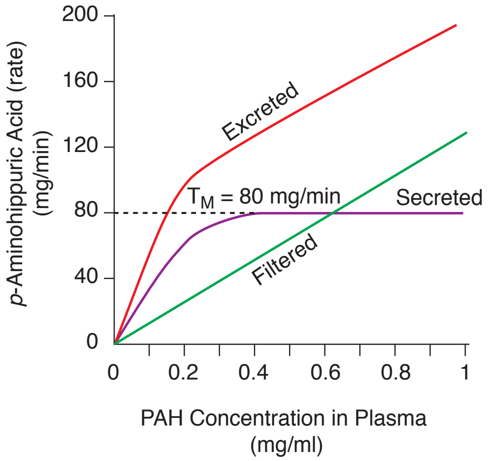
- 0.2 mg/mL gets filtered (filtration fraction of 20%).
- The remaining 0.8 mg/mL is secreted into the lumen, thus all 1 mg of PAH is now in the tubule.

What happens if the plasma concentration exceeds the TM for PAH?

Let's say the plasma concentration is 2 mg/mL. 0.4 mg is filtered (filtration fraction of 20%).

- If the TM for PAH is 1 mg/mL, then only 1 mg of PAH is added to the tubule; the remaining PAH (0.6 mg) leaves via the renal vein.
- Since only 1.4 mg of PAH is in the tubule, the clearance of PAH now underestimates ERPF.
- Further, as the plasma concentration increases, the clearance of PAH only increases in proportion to the rise in filtered load (~20% of the plasma PAH), but the plasma increases 80% (TM reached, so all "excess" PAH stays in the plasma). Thus, the higher the plasma PAH, the lower the clearance.

These points are summarized in the figure below..



Filtration: linear relationship with plasma concentration and represents 20% of PAH delivered to kidney

Secretion: initially 4× filtration rate and represents 80% of PAH delivered to kidney. Therefore, initially all the PAH delivered to kidney is removed—20% by filtration and 80% by secretion—and the concentration of PAH in the renal venous plasma should be zero. As plasma level rises, secretion increases, reaching maximum rate (T_M) when the carriers are saturated. PAH appears in renal venous plasma at the beginning of splay region in the secretion curve

Excretion: sum of the filtration rate and secretion rate. Once T_M is reached, increases in excretion parallel increases in filtration

Figure VI-2-6. Excretion of PAH

Transport of Organic Acids/Bases

PAH is transported by a fairly nonspecific organic anion transporter (OAT). Many compounds compete for the carriers. In addition to PAH, some of those compounds include:

- Penicillin
- Furosemide
- Acetazolamide
- Salicylate

Because the organic anions all compete for the same carriers, elevation of the plasma level of one ion inhibits the secretion and clearance of the others.

There is a similar transport secretory system for many organic cations. A slightly different transport mechanism is involved but, again, the system is fairly nonspecific. Drugs using this pathway include:

- Atropine
- Morphine
- Procainamide
- Cimetidine
- Amiloride

Note that, because of competition for the carrier proteins, the concurrent administration of organic cations can increase the plasma concentration of both drugs to much higher levels than when the drugs are given alone.

RENAL HANDLING OF SOME IMPORTANT SOLUTES

The illustrations below represent the net transport of specific types of substances for a normal individual on a typical Western diet (contains red meat). The dashed lines represent the route followed by the particular substance.

Quantitative aspects are not shown. For example, in B, 20% of the substance entering the kidney is filtered and excreted, and the remaining 80% passes through the kidneys and back into the general circulation without processing.

Illustrations meant to show overall net transport only

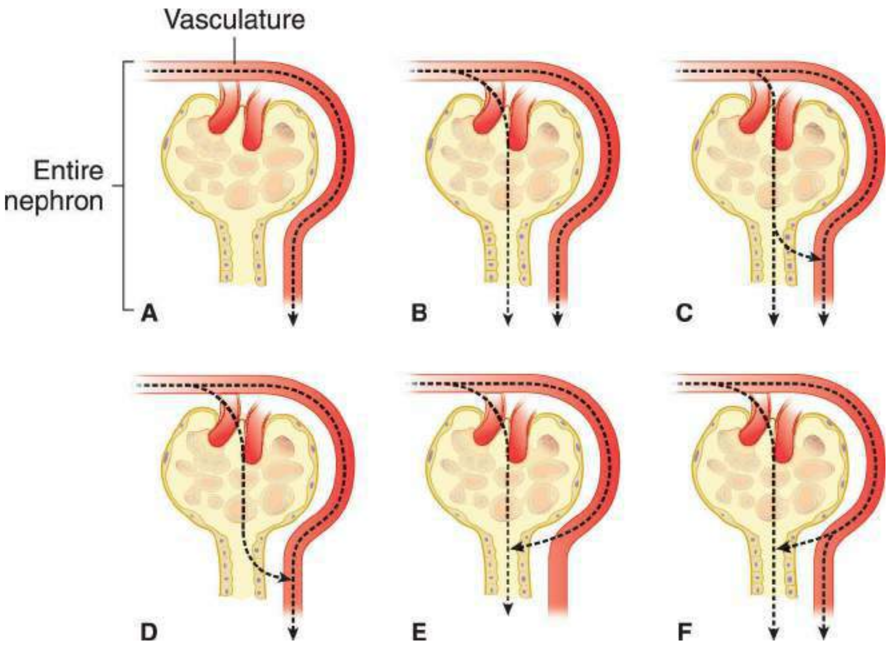


Figure VI-2-7. Graphical Representation of Transport

A: protein

B: inulin

C: potassium, sodium, urea

D: glucose, bicarbonate

E: PAH

F: creatinine

Clinical Estimation of GFR and Patterns of Clearance

3

Learning Objectives

- ❑ Use knowledge of clearance as an estimator of GFR
- ❑ Demonstrate understanding of clearance curves for some characteristic substances
- ❑ Solve problems concerning free water clearance
- ❑ Use knowledge of sodium and urea clearance

CLEARANCE AS AN ESTIMATOR OF GFR

Estimates of GFR are used clinically as an index of renal function and to assess the severity and the course of renal disease. A fall in GFR means the disease is progressing, whereas an increase in GFR suggests a recovery. In many cases a fall in GFR may be the first and only clinical sign of renal dysfunction. Estimations of GFR rely on the concept of clearance.

Substances having the following characteristics can be used to estimate GFR.

- Stable plasma concentration that is easily measured
- Freely filtered into Bowman's space
- Not reabsorbed, secreted, synthesized, or metabolized by the kidney

Ideal substances include inulin, sucrose, and mannitol. Even though the clearance of inulin is considered the gold standard for the measurement of GFR, it is not used clinically. Instead clinical estimates of GFR rely on creatinine.

Creatinine is released from skeletal muscle at a constant rate proportional to muscle mass. Muscle mass decreases with age but GFR also normally decreases with age. Creatinine is freely filtered and not reabsorbed by the kidney, though a very small amount is secreted into the proximal tubule.

Creatinine production = creatinine excretion =
filtered load of creatinine = Pcr × GFR

Thus, if creatinine production remains constant, a decrease in GFR increases plasma creatinine concentration, while an increase in GFR decreases plasma creatinine concentration.

Plasma creatinine, however, is not a very sensitive measure of reduced GFR. It only reveals large changes in GFR. As seen below, a significant reduction of GFR produces only a modest elevation of plasma or serum creatinine concentration.

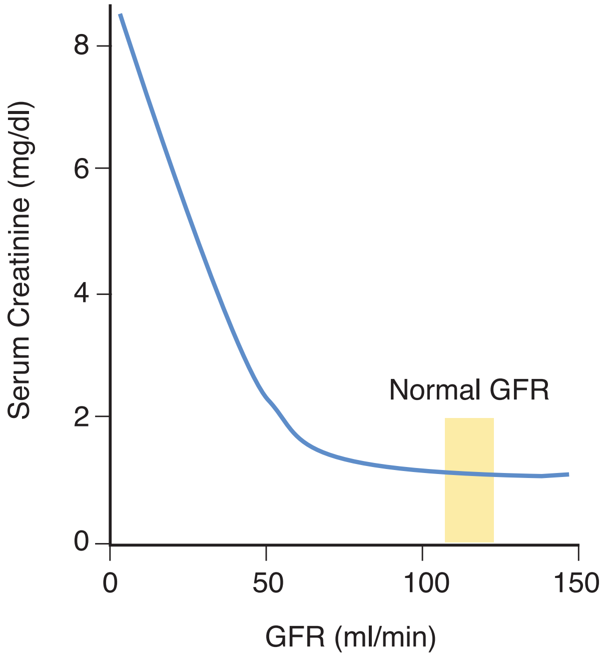


Figure VI-3-1. Serum Creatinine as Index of GFR

The only practical numerical estimate is the calculated clearance of creatinine. The following is all that is needed:

- Plasma creatinine concentration
- Timed collection of urine and the urine concentration of creatinine

CLEARANCE CURVES FOR SOME CHARACTERISTIC SUBSTANCES

The figure below plots clearance versus increasing plasma concentration for 4 substances. A description of each curve follows.

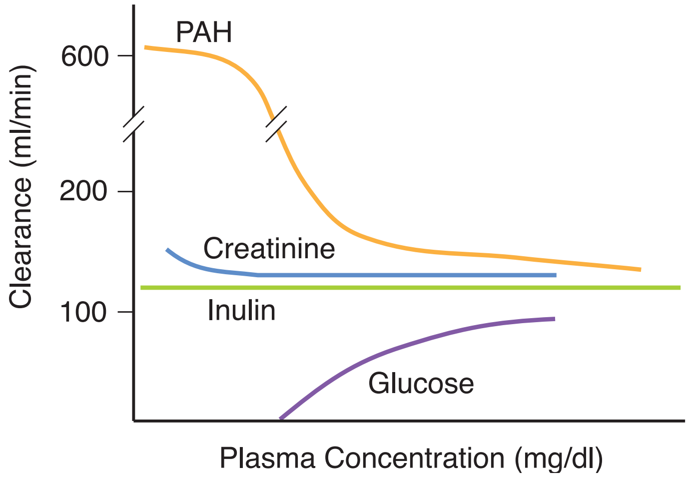


Figure VI-3-2. Clearance Curves

Inulin

- The clearance of inulin is independent of the plasma concentration, thus plotting it on the graph produces a line parallel to the X-axis. This is because a rise in the plasma concentration produces a corresponding rise in filtered load and thus a corresponding rise in ER (recall that inulin is neither secreted nor reabsorbed). In other words, the numerator and denominator of the clearance equation for inulin change in proportion, leaving the quotient (clearance) unchanged.
- It is always parallel to the x axis, and the point of intersection with the y axis is always GFR.
- If GFR increases, the line shifts upward; likewise, if GFR decreases, the line shifts down.

Glucose

- At low plasma levels, the clearance of glucose is zero because all of the FL is reabsorbed.
- As the plasma levels rise, the FL exceeds the TM in some nephrons and as a result, glucose appears in the urine and thus has a clearance.
- The plasma level at which glucose first appears in the urine is called the plasma (or renal) threshold.
- As the plasma level rises further, the clearance increases and approaches that of inulin. The clearance never equals inulin because some glucose is always reabsorbed.

Creatinine

- Because there is some secretion of creatinine, the clearance is always greater than the clearance of inulin.
- However, because only a small amount is secreted, creatinine clearance parallels inulin clearance and is independent of production rate (excretion rises as plasma concentration increases).
- Because it is endogenously produced, it is not necessary to infuse it to get a clearance measurement, as has to be done to measure inulin clearance. Therefore, the clearance of creatinine is the preferred clinical method for determining GFR (see above).

PAH

- At low plasma concentrations, the clearance equals renal plasma flow.
- As the plasma concentration rises, the carriers in some nephrons hit TM, resulting in some PAH appearing in the renal venous plasma.
- Plasma concentrations above TM reduce the clearance of PAH (described in chapter 2)
- As the plasma level rises further, the clearance approaches but never equals GFR because some PAH is always secreted.

Summary of the highest clearance to the lowest clearance:

PAH > creatinine > inulin > urea > sodium > glucose = albumin

Remember, if it is in the renal vein, it is not cleared. This could be because it was not filtered (like albumin) or it was filtered and all reabsorbed (like glucose).

FREE WATER CLEARANCE

Free water clearance is the best measure of the balance between solute and water excretion. Its use is to determine whether the kidneys are responding appropriately to maintain normal plasma osmolality. Free water clearance is how much solute-free water is being excreted; it is as if urine consisted of plasma (with solutes) plus or minus pure water.

- If urine osmolality is 300 mOsm/kg (isotonic urine), free water clearance is zero.
- If plasma osmolality is too low, urine osmolality should be lower still (positive free water clearance) in order to compensate.
- Positive-free water clearance tends to cause increased plasma osmolality; negative free water clearance causes reduced plasma osmolality.
- C_{H2O} (+) = hypotonic urine is formed (osmolality <300 mOsm/kg)
- C_{H2O} (–) = hypertonic urine is formed (osmolality >300 mOsm/kg)

V: urine flow rate

U_{osm}: urine osmolarity

P_{osm}: plasma osmolarity

$$C_{H_2O} = V - \frac{U_{osm}V}{P_{osm}}$$

$$V = C_{H_2O} + C_{osm}$$

Sample Calculation

$\dot{V} = 3.0$ mL/min

$U_{osm} = 800$ mOsm/L

$P_{osm} = 400$ mOsm/L

$C_{H_2O} = -3$ mL/min

Conclusion: The kidneys are conserving water; this is appropriate compensation for the excessive plasma osmolarity in this patient.

SODIUM AND UREA CLEARANCE

Sodium

Sodium always appears in the urine, thus sodium always has a positive clearance.

- The fractional excretion of Na^+ (F_ENa^+; equation not shown) indicates the fraction (percentage) of the filtered Na^+ that is excreted. It is very useful in differentiating prerenal from intrarenal acute renal failure (see next chapter).

- Since almost the entire filtered load of sodium is reabsorbed its clearance is just above zero. Aldosterone, by increasing the reabsorption of sodium, decreases the $FeNa^+$. Atrial natriuretic peptide (ANP) increases the $FeNa^+$ because it causes a sodium diuresis.

Urea

Urea is freely filtered but partially reabsorbed. Because some urea is always present in the urine, you always clear a portion of the 120 mL/min filtered into Bowman's space.

- Since urea tends to follow the water and excretion is flow dependent, a diuresis increases urea clearance and an antidiuresis decreases urea clearance.
- ADH increases reabsorption of urea in the medullary collecting duct → increase in BUN → decrease in clearance → if the plasma concentration is increasing in the renal venous plasma, less is cleared from the plasma
- With a small volume of concentrated urine, the concentration of urea is relatively high, but the excretion is less than in a diuresis that has a much lower concentration of urea. It is the large volume in the diuresis that increases the urea excretion and clearance.

Recall Question

Which of the following is a characteristic of inulin?

A. As the plasma levels of inulin rise, the FL exceeds the TM in some nephrons and inulin appears in the urine

B. The clearance of inulin is the preferred clinical method for determining RBF

C. The clearance of inulin is independent of the plasma concentration

D. As plasma inulin rises, the clearance approaches but never equals GFR because some inulin is always secreted

E. Inulin is reabsorbed with sodium via the renin angiotensin aldosterone system

Answer: C

Regional Transport 4

Learning Objectives

- ❑ Solve problems concerning the proximal tubule
- ❑ Explain information related to loop of Henle
- ❑ Use knowledge of distal tubule
- ❑ Use knowledge of collecting duct
- ❑ Answer questions about renal tubular acidosis
- ❑ Explain information related to disorders of potassium homeostasis
- ❑ Demonstrate understanding of renal failure

PROXIMAL TUBULE

The fluid that enters the proximal tubule is an isotonic ultrafiltrate (300 mOsm/kg). The concentration of a freely filtered substance in this fluid equals its plasma concentration. The main cellular transport processes of the proximal tubular cells can be seen below.

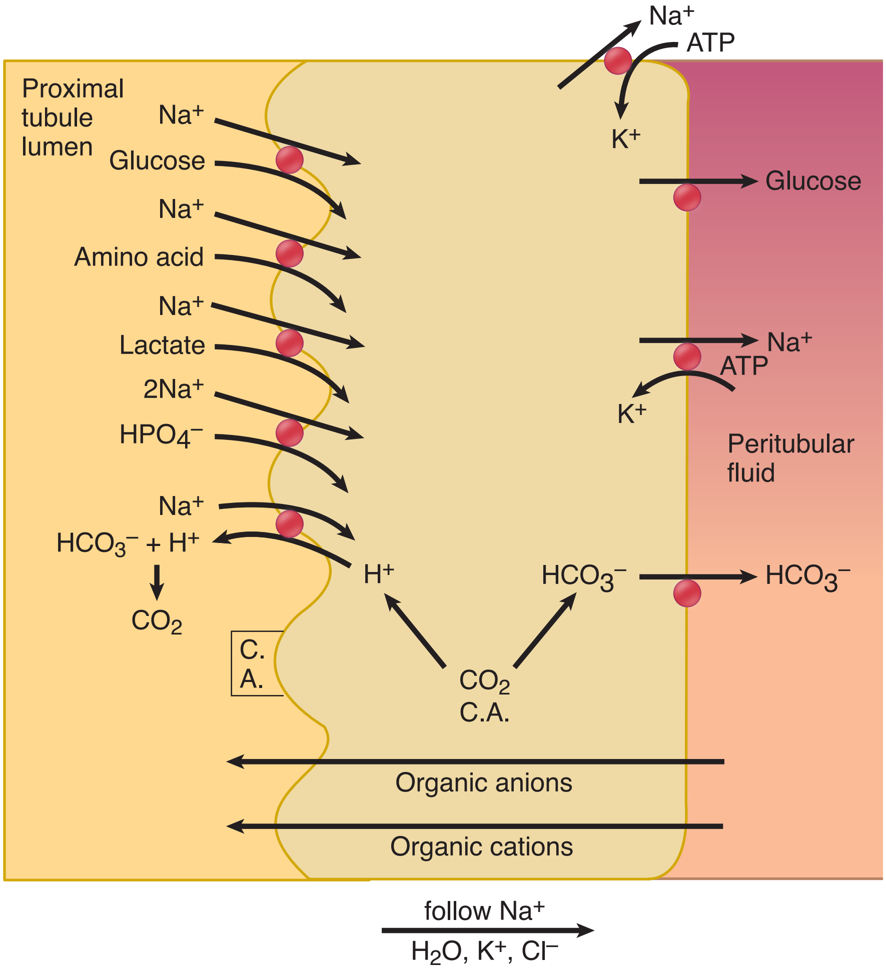


Figure VI-4-1. Transport in Proximal Tubule

Proximal Tubule Changes

Sodium

- Approximately two-thirds of the filtered sodium is reabsorbed in the proximal tubule (PT). The basolateral Na^+--K^+ ATPase creates the gradient for Na^+ entry into the cell and its removal from the cell back into the bloodstream.
- Although it can be modified some, the PT captures two-thirds of the filtered sodium (referred to as glomerulotubular balance). Recapturing two-thirds of the sodium helps protect extracellular volume despite any changes that may occur in GFR.
- Catecholamine and angiotensin II stimulate the basolateral ATPase and thus enhance the fraction of sodium reabsorbed in the proximal tubule.

Water and electrolytes

- About two-thirds of the filtered H_2O, K^+ and almost two-thirds of the filtered Cl^- follow the sodium (leaky system to these substances), and the osmolality at the end of the proximal tubule remains close to 300 mOsm/kg (isosmotic reabsorption). The chloride concentration rises slightly through the proximal tubule because of the large percentage of bicarbonate reabsorbed here.
- Therefore, at the end of the proximal tubule, osmolality and the concentrations of Na^+ and K^+ have not changed significantly from plasma, but only one-third of the amount originally filtered remains.

Metabolites

- Normally, all of the filtered glucose is reabsorbed in the PT via secondary active transport linked to sodium. This transporter is termed the sodium glucose-linked transporter (SGLT) and type 2 (SGLT-2) is the predominant form in the kidney.
- In addition, all proteins, peptides, amino acids, and ketone bodies are reabsorbed here via secondary active transport (requires luminal sodium, linked to sodium reabsorption).
- Therefore, the concentration of the above should be zero in the tubular fluid leaving the proximal tubule (clearance is zero).

Bridge to Pharmacology

SGLT-2 blocker canagliflozin **inhibits** proximal tubule reabsorption of glucose and is used to treat type 2 DM.

Bicarbonate

About 80% of the filtered bicarbonate is reabsorbed here. The mechanism for this reabsorption is:

- Bicarbonate combines with free H^+ in lumen and is converted into CO_2 and H_2O, catalyzed by the luminal carbonic anhydrase enzyme (CA). H^+ is pumped into the lumen in exchange with sodium (antiporter). Although not pictured, there is a H^+--ATPase on the luminal membrane that contributes to pumping H^+ into the lumen.
- CO_2, being very soluble, crosses the luminal membrane where it combines with water to reform H^+ and bicarbonate (note CA in the cell). The H^+ is then pumped back into the lumen, while bicarbonate exits the baslolateral membrane to complete its reabsorption.
- Because of this mechanism, bicarbonate reabsorption is dependent upon H^+ secretion and the activity of CA.
- The most important factor for H^+ secretion is the concentration of H^+ in the cell. Thus, H^+ secretion and bicarbonate reabsorption are increased during an acidosis and they decrease with an alkalosis.
- Angiotensin II stimulates the Na^+--H^+ antiporter. Thus, in volume-depleted states, the amount of bicarbonate reabsorption in the PT increases. This is thought to be the mechanism preventing bicarbonate loss when a patient develops a contraction alkalosis.

The small amount of bicarbonate that leaves the proximal tubule is normally reabsorbed in subsequent segments.

Bridge to Pharmacology

The primary site of action for carbonic anhydrase inhibitors is the PT. Blocking CA reduces bicarbonate reabsorption and the activity of the Na^+--H^+ exchanger.

Clinical Correlate

An 85-year-old woman presents to the emergency room with confusion. She is on hydrochlorothiazide. Her bicarbonate is 34 mEq/L (normal 24 mEq/L). What is the cause of the metabolic alkalosis?

This is a "contraction alkalosis." The thiazide diuretic is causing a decrease in the intravascular volume. You can see the same in sweating in the desert or vomiting (not diarrhea because you lose bicarbonate in the stools and get a metabolic acidosis). The low volume state increases renin secretion leading to high angiotensin II. Angiotensin II activates the sodium/hydrogen exchanger →, increasing the reabsorption of bicarbonate → maintaining the metabolic alkalosis.

Clinical Correlate

Administration of a solute that is freely filtered but not reabsorbed (mannitol) and/or reducing/preventing the normal reabsorption of a solute results in the osmotic pull of water into the lumen and diuresis occurs. From the standpoint of the PT, glucose exceeding the TM is an important example.

A: PAH

B: inulin

C: substance reabsorbed somewhat less rapidly than water, e.g., chloride

D: major electrolytes such as sodium, potassium

E: substance reabsorbed somewhat more rapidly than water

F: substance completely reabsorbed in proximal tubule, e.g., glucose

Urate (uric acid)

The details of the renal handling of urate are too complex for the scope of this book. In short:

- Urate is formed by the breakdown of nucleotides
- Xanthine oxidase is the enzyme that catalyzes the final reaction to form urate.
- About 90% of the filtered urate is reabsorbed by the proximal tubule.
- If the FL of urate is high enough and the luminal pH is low, then more of the urate exists as uric acid, which can precipitate and form a kidney stone.
  - This is not the most common type of kidney stone, but it can occur in patients with gout.

Secretion

The proximal tubule is where many organic anions and cations are secreted and cleared from the circulation including PAH, penicillin, atropine, and morphine.

Energy requirements

Notice above that all of the active processes are powered by the Na/K-ATPase primary active pump. This pump is located in the proximal tubule basal and basolateral borders and is directly or indirectly responsible for most of the water and electrolyte reabsorption in the nephron. It thus represents the most energy-demanding process of the nephron.

The figure below depicts the ratio of the concentration of the substance in the proximal tubular fluid (TF) to the concentration in the plasma (P), beginning in Bowman's space through the end of the PT.

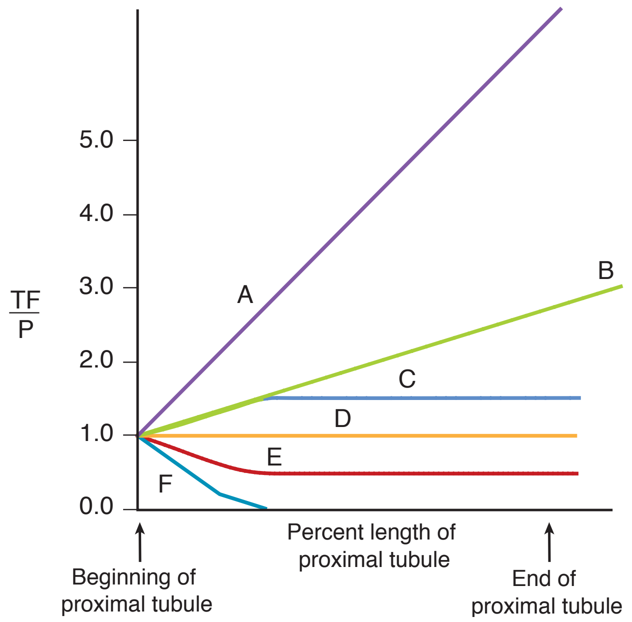


Figure VI-4-2. Proximal Tubule Transport

Concentration of Inulin in the Nephron Tubule

The concentration of inulin along the nephron tubule is an index of water reabsorption.

- Inulin is freely filtered; thus, its concentration in Bowman's space is the same as it is in the plasma. Because water is reabsorbed but inulin is not, the concentration of inulin increases throughout the nephron. The greater the water reabsorption, the greater the increase in inulin concentration.
- Since two-thirds of the water is reabsorbed in the proximal tubule, the inulin concentration should triple TF/P = 3.0. Its concentration should further increase in the descending limb of the loop of Henle, distal tubule, and the collecting duct (assuming ADH is present).
- The segment of the nephron with the highest concentration of inulin is the terminal collecting duct. The segment of the nephron with the lowest concentration of inulin is Bowman's space.

LOOP OF HENLE

Fluid entering the loop of Henle is isotonic (300 mOsm/kg), but the volume is only one-third the volume originally filtered into Bowman's space.

- The loop of Henle has countercurrent flow and it acts as a countercurrent multiplier, the details of which are not imperative to learn. In short, the loop of Henle creates a concentrated medullary interstitium.
- The osmolality of the medulla can reach a maximum of about 1200 mOsm/kg, and the predominant osmoles are NaCl and urea (see Figure VI-4-3).
- Juxtamedullary nephrons are responsible for this extremely high medullary osmolality. They are surrounded by vasa recta and slow flow in the vasa recta is crucial for maintaining the concentrated medullary interstitium.

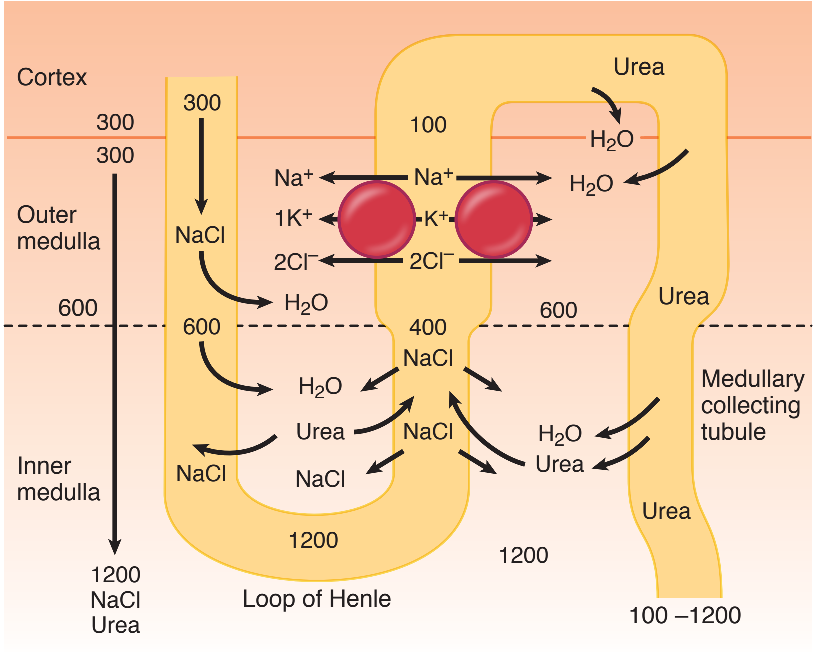


Figure VI-4-3. Countercurrent and the Loop of Henle

Clinical Correlate

For a patient who has diarrhea, vomiting, or hemorrhaging, it is important to preserve extracellular volume; one way to do so is to increase reabsorption of fluid and electrolytes at the proximal tubules.

Clinical Correlate

In nephrogenic diabetes insipidus (DI), ADH receptors are not functioning and it is not possible to increase reabsorption at the collecting duct. The patient loses free water and develops hypernatremia. Treatment is reduction of extracellular volume with a thiazide diuretic. This increases peritubular oncotic pressure, in turn increasing water reabsorption in the proximal tubule. The elevated water reabsorption, along with sodium loss in the urine (action of thiazide diuretics), corrects the hypernatremia.

Clinical Correlate

A 65-year-old man presents with hyponatremia. His serum osmolarity is low and urine osmolarity high. He is diagnosed with SIADH. He is started on fluid restriction but is not able to comply. He is started on a loop diuretic, and plasma sodium increases.

Loop diuretics decrease the reabsorption of sodium and chloride at the loop of Henle. This removes the concentrating effect of the loop of Henle, decreasing the osmolar gradient. This, in turn, decreases the reabsorption for free water from the collecting duct.

Descending Limb

- Permeable to water (about 15% of filtered water is reabsorbed here)
- Relatively impermeable to solute

Ascending Limb

- Impermeable to water
- Solutes transported out

A typical cell in the ascending thick limb (ATL) of the loop of Henle can be seen below. Similar to the PT, there is a luminal Na^+--H^+ antiporter and bicarbonate is reabsorbed here.

Na^+--K^+--$2Cl^-$ transporter

This is an electroneutral transport resulting in the reabsorption of about 25% of the filtered sodium, chloride, and potassium.

- The luminal membrane contains a K^+ channel (Figure VI-4-4), allowing diffusion of this ion back into lumen (recall that the concentration of K^+ inside cells is very high compared to the extracellular concentration).
- This back diffusion of K^+ into the lumen creates a positive luminal potential, which in turn, promotes calcium and magnesium reabsorption (about 25% of FL) via a paracellular pathway (primarily). This positive luminal potential also causes sodium reabsorption via a paracellular pathway.

Calcium-sensing receptor (CaSR)

The basolateral membrane of cells in ATL contain CaSR (Figure VI-4-4), which is a G-protein coupled receptor. Because it is on the basolateral membrane, CaSR is influence by the plasma concentration of calcium.

- CaSR couples to at least two G-proteins: 1) $G_{I/O}$, which inhibits adenylyl cyclase, thereby reducing intracellular cAMP, and 2) G_q, which activates protein kinase C (PKC). The net effect of these changes in intracellular signaling pathways is an inhibition of the Na^+--K^+--$2Cl^-$ transporter.
- Reducing the activity of the Na^+--K^+--$2Cl^-$ transporter reduces the positive luminal potential (less K+ back diffusion), which in turn, decreases calcium reabsorption.
- Thus, high plasma concentrations of calcium can directly reduce calcium reabsorption in ATL.

Bridge to Pharmacology

Loop diuretics block the Na^+--K^+--$2Cl^-$ transporter in ATL, thereby reducing their reabsorption. Blocking this transporter also reduces calcium and magnesium reabsorption, all of which results in a marked diuresis.

Bridge to Pathology

Bartter syndrome is a genetic mutation resulting in diminished function of the Na^+--K^+--$2Cl^-$ transporter in ATL. This leads to a low volume state, which causes an increase in renin and aldosterone (known as a secondary hyperaldosteronism). Patients exhibit hypokalemia, alkalosis, and elevated urine calcium.

Bridge to Pathology

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant genetic disorder resulting in hypercalcemia.

- The CaSR is mutated such that it does not respond to plasma calcium; the CaSR is inactive and "fooled" into thinking that the plasma calcium is low when it is in fact elevated.
- Thus, calcium reabsorption in the kidney is elevated despite the hypercalcemia.
- Patients also have high levels of parathyroid hormone (PTH) because CaSR is expressed in cells of the parathyroid gland. The mutation prevents the inhibition of PTH that normally occurs in response to hypercalcemia.

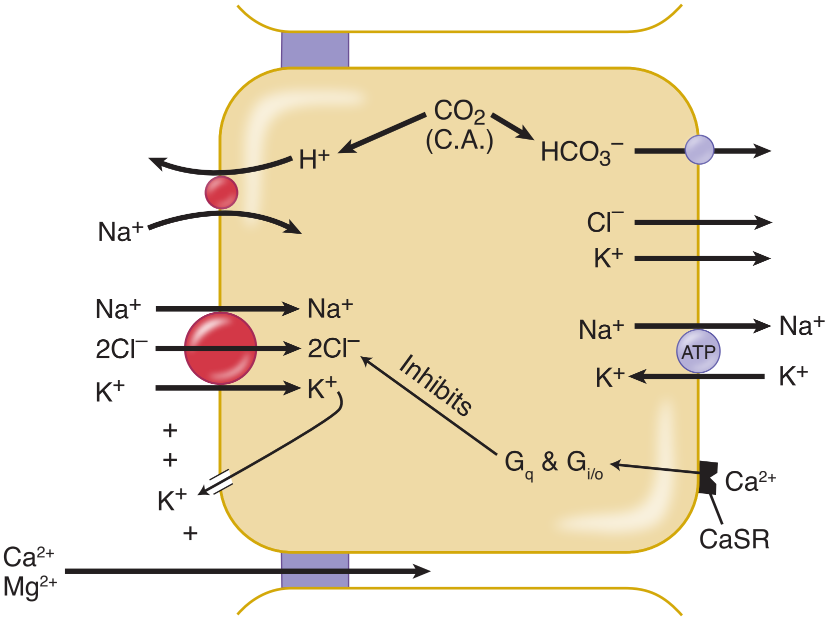


Figure VI-4-4. Loop of Henle

Recall Question

In which part of the nephron is ampicillin most likely to be secreted and cleared from the circulation?

A. Proximal tubule

B. Descending limb of loop of Henle

C. Ascending limb of loop of Henle

D. Distal tubule

E. Collecting duct

Answer: A

DISTAL TUBULE

The early distal tubule reabsorbs Na^+, Cl^-, and Ca^{2+}.

NaCl

NaCl crosses the apical membrane via a Na^+-Cl^- symporter.

- The Na^+ is pumped across the basal membrane via the Na/K-ATPase proteins and Cl^- diffuses down its electrochemical gradient through channels.
- This section is impermeable to water. Thus, osmolality decreases further. In fact, the ultrafiltrate in the early distal tubule has the lowest osmolality of the entire nephron.

Bridge to Pharmacology

Thiazide diuretics block the NaCl symporter in the distal tubule. Blocking this transporter enhances calcium reabsorption in the distal tubule and can result in hypercalcemia. In addition, thiazide diuretics are sometimes used to increase plasma calcium.

Bridge to Pathology

Gitelman syndrome is a genetic disorder resulting in a mutated (reduced function) NaCl transporter. These patients are hypokalemic, alkalotic, and have a low urine calcium.

Calcium

Calcium enters the cell from the luminal fluid passively through calcium channels. The opening of these channels is primarily regulated by parathyroid hormone (PTH).

- Calcium is actively extruded into the peritubular fluid via Ca^{2+}-ATPase or a $3Na^+$-Ca^{2+} antiporter.
- These cells also express the calcium binding protein, calbindin, which facilitates calcium reabsorption. Calbindin synthesis is increased by the active form of vitamin D, and thus vitamin D enhances PTH's action on the distal tubule.

Cal: calbindin

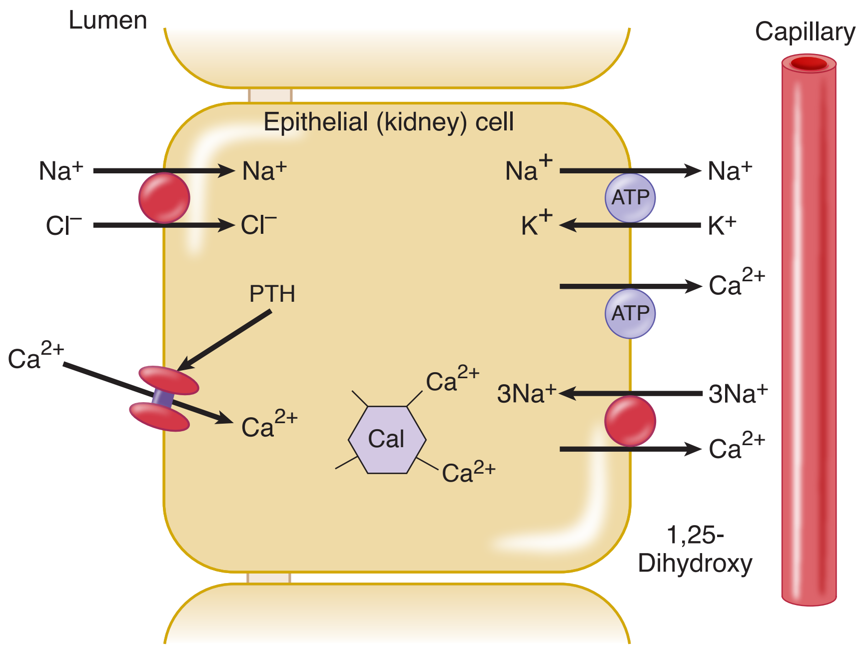


Figure VI-4-5. Transporters in Distal Tubule

COLLECTING DUCT

The collecting duct (CD) is composed of principal cells and intercalated cells.

Principal Cells

- The luminal membrane of principal cells contains sodium channels, commonly referred to as epithelial Na^+ channels (ENaC). Because of these channels, sodium follows its electrochemical gradient (created by the basolateral Na^+--K^+ ATPase) into the cell.
- Some chloride does not follow the sodium, thus the reabsorption of sodium produces a negative luminal potential. This negative luminal potential causes potassium secretion.
- Thus, the reabsorption of sodium and secretion of potassium are linked.

- Mineralocorticoids such as aldosterone exert an important effect on these cells. Activation of the mineralocorticoid receptor increases the number of luminal ENaC channels, increases their open time, and induces synthesis and trafficking of the basolateral Na^+–K^+ ATPase. The net effect is increased sodium reabsorption and potassium secretion.
- Although not illustrated on the slide, principal cells express aquaporins, which are regulated by anti-diuretic hormone (ADH), also known as arginine vasopressin (AVP). ADH acts on V2 (Gs—cAMP) receptors to cause insertion of aquaporins, which in turn, causes water (and urea) reabsorption.

Intercalated Cells

- Intercalated cells are intimately involved in acid-base regulation. The amount of fixed acid generated by an individual is mainly determined by diet. A high percentage of animal protein in the diet generates more fixed acid than a vegetarian-based diet.
- The luminal membrane contains a H^+–ATPase, which pumps H^+ into the lumen. Although free H^+ is pumped into the lumen, luminal pH can only go so low before it causes damage to cells, and thus most of the H^+ is eliminated from the body via buffers, phosphate and ammonia being the two most common.
- Monoprotonated phosphate is freely filtered at the glomerulus. About 80% is reabsorbed in the PT and another 10% is reabsorbed in the distal tubule. The remaining phosphate serves as a buffer for the secreted H^+. The H^+ pumped into the lumen binds to phosphate to form diprotonated phosphate, which is poorly reabsorbed, thus eliminating H^+ from the body. Phosphate is the primary titratable acid.
- In addition, the H^+ pumped into the lumen can combine with ammonia to form ammonium, which is not reabsorbed and is thus excreted. Ammonia is produced by the catabolism of glutamine and this occurs in cells of the PT. Ammonia synthesis and secretion in the proximal tubule **increases in response to an acidosis** and **decreases in response to an alkalosis** in proximal tubule cells.
- For every H^+ excreted by the above buffers, bicarbonate is added to the body (new bicarbonate).
- Aldosterone stimulates the H^+--ATPase of intercalated cells. Thus, excess aldosterone results in a metabolic alkalosis.

Bridge to Pharmacology

Potassium sparing diuretics work by blocking ENaC, e.g., amiloride, or by blocking aldosterone receptors, e.g., spironolactone, or the production of aldosterone, e.g., blockers of the RAAS system. Because sodium reabsorption is reduced, potassium secretion is diminished.

Bridge to Pathology

Liddle syndrome is a genetic disorder resulting in a gain of function of ENaC channels in the CD.

This results in enhanced sodium reabsorption and potassium secretion. Patients are hypertensive, hypokalemic, and alkalotic.

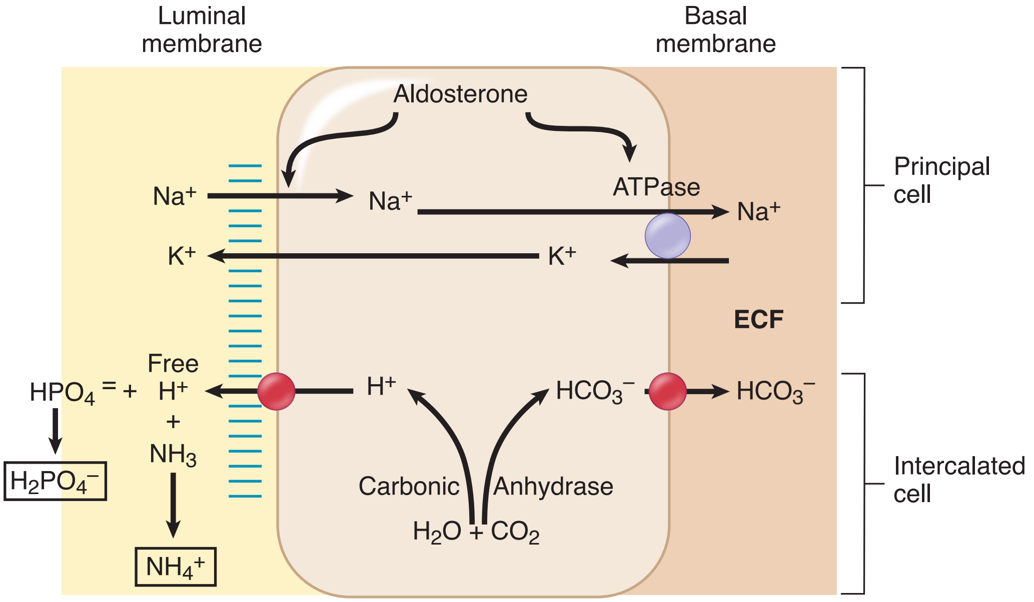


Figure VI-4-6. Late Distal Tubule and Collecting Duct

RENAL TUBULAR ACIDOSIS

Proximal Renal Tubular Acidosis (Type II)

Proximal renal tubular acidosis (type II) is the result of a diminished capacity of the proximal tubule to reabsorb bicarbonate.

- Transient appearance of bicarbonate in the urine until the filtered load is reduced to match the reduced capacity of reabsorption.
- Steady-state characterized by a low plasma bicarbonate and acid urine.
- An example would be Fanconi syndrome, which involves a general defect in the proximal tubular transport processes and carbonic anhydrase inhibitors.
- Serum potassium is also low. When bicarbonate is lost in the urine, it is lost as sodium bicarbonate and that pulls water with it creating an osmotic diuresis. The diuresis leads to loss of potassium in the urine.

Distal Renal Tubular Acidosis (Type I)

Distal renal tubular acidosis (type I) is the result of an inability of the distal nephron to secrete and excrete fixed acid, thus an inability to form an acid urine. Urine pH >5.5 – 6.0.

- Mechanisms would include impairment of the transport systems for hydrogen ions and bicarbonate and an increased permeability of the luminal membrane allowing the back diffusion of the hydrogen ions from the tubular lumen.
- The result is a metabolic acidosis with an inappropriately high urine pH.
- Serum potassium is also low.

Causes include autoimmune disorders (e.g., lupus, sarcoidosis, Sjogren); lithium; and hypercalciuric conditions (calcium-damaging cells).

Renal Tubular Acidosis Type IV: Hypoaldosterone States

Renal tubular acidosis type IV is the result of an inability to secrete potassium, leading to hyperkalemia. There is decreased secretion of protons, leading to metabolic acidosis.

Causes:

- Diabetic nephropathy, due to low renin secretion with loss of kidney function
- Any drug that inhibits the RAAS system, such as ACE-inhibitors, ARBs, spironolactone, and aliskiren
- Trimethoprim
- Addison disease due to loss of aldosterone secretion from the adrenal cortex

DISORDERS OF POTASSIUM HOMEOSTASIS

Potassium Balance

To keep the body amount constant, excretion of potassium must match dietary intake, and the kidneys regulate potassium excretion. A small percentage of ingested potassium is lost in the stool but this is not a major regulatory route under normal conditions.

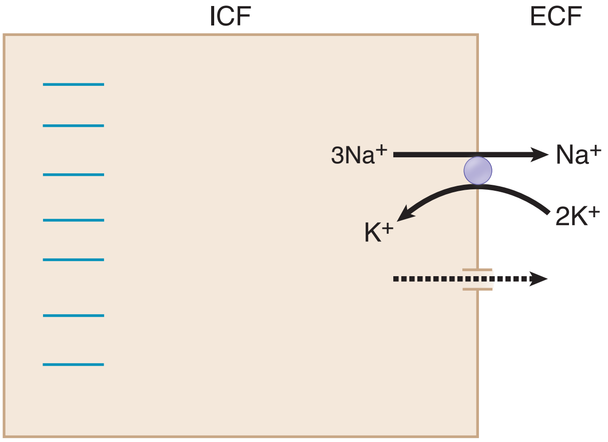


Figure VI-4-7. ICF and ECF Potassium Distribution

- 98% of potassium inside cells
- 2% of potassium in ECF (4 mEq/L)
- >5.0 mEq/L = hyperkalemia
- <3.5 mEq/L = hypokalemia

- Insulin and epinephrine stimulate the Na/K ATPase and can thus reduce plasma potassium.
- Long term balance is maintained via aldosterone's effect on potassium secretion in the distal tubule and collecting ducts of the nephrons.
- Acidosis and increased ECF osmolality (cell shrinkage) shifts potassium from the ICF to the ECF. Inorganic fixed acid > organic acids > respiratory acidosis
- Alkalosis and decreased ECF osmolality (cell swelling) shifts potassium from the ECF to the ICF. Metabolic alkalosis > respiratory alkalosis

Potassium secretion and excretion by the kidney

Potassium secretion is determined mainly by 2 factors: filtrate flow and sodium reabsorption (creates negative potential of the lumen).

- Increased flow and/or aldosterone increases potassium secretion and excretion.
- Decreased flow and/or aldosterone decreases potassium secretion and excretion.

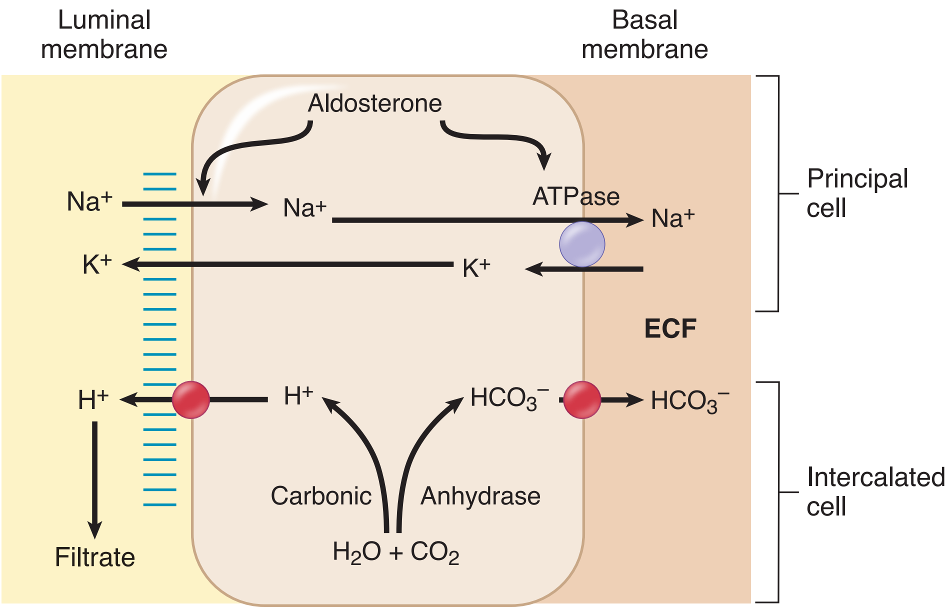


Figure VI-4-8. Late Distal Tubule and Collecting Duct

Acid-Base Disorders

Acidosis shifts potassium from the ICF to ECF. Decreased intracellular potassium reduces the potassium gradient, thus potassium secretion falls (both promote hyperkalemia).

The negative potassium balance in acidosis is not typically sustained, however, because hyperkalemia stimulates aldosterone.

Alkalosis shifts potassium from ECF to ICF. Increased intracellular potassium increases potassium secretion (both promote hypokalemia).

Summary of Potassium Balance

Promoters of hyperkalemia

- Transcellular shifts: metabolic acidosis, hyperglycemia, insulin deficiency or resistance, muscle trauma
- GI: excessive intake (on rare occasions)
- Kidney: acute oligouric kidney disease, chronic kidney disease where GFR decreases dramatically from normal, hypoaldosteronism

Consequences of hyperkalemia

- Neuromuscular function: muscle weakness, general fatigue if chronic
- Cardiac: high T wave, eventually in severe hyperkalemia ventricular fibrillation
- Metabolic: metabolic acidosis

Promoters of hypokalemia

- Transcellular shifts: metabolic alkalosis, sudden increases in insulin and catecholamines
- GI: diarrhea, vomiting, low potassium diet (rarely has an effect on its own)
- Kidney: diuretics due to increased flow (thiazides, loop diuretics, osmotic diuresis), hyperaldosteronism (adrenal adenoma, renal arterial stenosis), increased excretion of negative ions (bicarbonate, ketone bodies), renal tubular acidosis types I and II.

Consequences of hypokalemia

- Neuromuscular function: muscle weakness, general fatigue
- Cardiac: hyperpolarization affects excitability and delays repolarization.
- EKG effects: low T wave, high U wave
- Metabolic: decreased insulin response to carbohydrate load, decreased growth rate in children, nephrogenic diabetes insipidus, metabolic alkalosis

RENAL FAILURE

Acute Renal Failure

Acute renal failure is a rapid loss of renal function that is often reversible. Loss of renal function results in the accumulation of waste products that the kidney excretes, e.g., BUN and creatinine. Depending upon the cause, the fractional excretion of Na^+ ($FeNa^+$) is either elevated or reduced. As its name implies, $FeNa^+$ simply means the % of filtered Na^+ that is excreted: The higher the number, the less reabsorption and vice versa. The causes of acute renal failure are:

Clinical Correlate

A 55-year-old woman with a history of end-stage renal failure presents with confusion after missing a dialysis session. Her potassium is elevated. While waiting for the nurse to set up the dialysis, the patient is treated with an injection of bicarbonate and insulin with dextrose.

Giving bicarbonate gives the patient an alkalosis. This causes protons to leave the intracellular space down its concentration gradient. To maintain electroneutrality, potassium shifts into cells. This decreases the extracellular potassium concentration. Insulin activates the sodium/potassium ATPase and that increases the shift of potassium into the cell also.

Prerenal

With prerenal renal failure, there is decreased renal perfusion as would occur with a decreased renal perfusion pressure, e.g., hypovolumia of hemorrhage, diarrhea, vomiting; congestive heart failure. Initially, there is no renal injury and it is reversible if corrected early. Characteristic signs are:

- Reduced GFR
- Reduced $FeNa^+$: Tubular function is intact and the low GFR (reduced filtered load) allows for significant reabsorption.
- Na^+ reabsorption: In addition, Ang II and catecholamines are often elevated in this condition, both of which increase Na^+ reabsorption.
- Elevated plasma BUN:Cr: Although both are elevated, the high reabsorption of urea (water reabsorption is elevated in prerenal) elevates BUN more than creatinine.

Intrarenal

In this condition, tubular damage occurs resulting in tubular dysfunction. Toxins, interstitial nephritis, ischemia, rhabdomyolysis, and sepsis are factors that could cause acute tubular necrosis and intrarenal failure. Characteristic signs are:

- Increased $FeNa^+$: Tubules are damaged and thus unable to reabsorb Na^+
- Casts/cells in the urine: Damaged cells are sloughed off into the tubule
- Low plasma BUN:Cr: Tubular damage prevents reabsorption of urea

Postrenal

This condition is caused by obstruction of fluid outflow from the kidney, e.g., renal calculi, enlarged prostate.

- Early: Characteristics are similar to prerenal, i.e., reduced $FeNa^+$, elevated plasma BUN:Cr
- Late: Buildup of pressure results in tubular damage, resulting in characteristics of intrarenal failure, i.e., marked increase in $FeNa^+$, low plasma BUN:Cr

Chronic Renal Failure

Although nephrons often recover from the sloughing of the tubular epithelial cells in acute renal failure, in chronic renal failure there is an irreversible loss of nephrons. To compensate, the remaining nephrons have an increased glomerular capillary pressure and hyperfiltration.

One way to look at this is a “hypertension” at the level of the nephron; the hyperfiltration combined with the increased work load promotes further injury leading to fibrosis, scarring, and loss of additional nephrons.

Looking back at the function of the kidney and how it regulates a variety of physiologic variables, many of the consequences of chronic renal failure are predictable. These include:

- Inability to excrete waste products leads to a rise in plasma BUN and creatinine.
- Inability to regulate sodium and water: This can lead to hyponatremia, volume overload, and thus edema. In addition, patients are susceptible to rapid development of hypernatremia and volume depletion following vomiting and diarrhea.
- Inability to regulate potassium excretion leads to hyperkalemia.
- Inability to excrete fixed acids leads to metabolic acidosis (elevated anion gap—see next chapter)
- Inability to excrete phosphate leads to hyperphosphatemia. This hyperphosphatemia reduces plasma calcium, causing a rise in parathyroid hormone (PTH—secondary hyperparathyroidism) resulting in increased bone resorption (renal osteodystrophy).
- Inability to hydroxylate (1-alpha hydroxylase enzyme is in the kidney) 25,OH-cholecalciferol decreases circulating levels of active vitamin D, which contributes to the hypocalcemia.
- Inability to secrete erythropoietin results in anemia.

The most common cause of chronic renal failure is the nephropathy produced by diabetes. The second most common cause is hypertension.

Recall Question

Which of the following medications causes an intracellular shift of potassium?

A. IV dextrose

B. IV insulin

C. Propranolol

D. Acetazolamide

E. Digoxin

Answer: B

Acid–Base Regulation 5

Learning Objectives

- ❑ Interpret scenarios on buffering systems
- ❑ Explain information related to formulating a diagnosis
- ❑ Explain information related to 3-question method
- ❑ Solve problems concerning the 4 primary disturbances
- ❑ Use knowledge of compensation
- ❑ Solve problems concerning plasma anion gap diagnosis
- ❑ Use knowledge of graphical representation (Davenport plot)
- ❑ Solve problems concerning supplemental information

BUFFERING SYSTEMS

The CO_2-bicarbonate buffer system can be seen below. It is one of the major buffer systems of the blood, and the one we focus on in this chapter.

$$H_2O + CO_2 \underset{}{\overset{CA}{\rightleftarrows}} H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

Figure VI-5-1. Production of Carbonic Acid

To demonstrate the changes in the major variables during acid–base disturbances, the scheme can be simplified to the following:

$$CO_2 \longleftrightarrow H^+ + HCO_3^-$$

Recall that the respiratory system plays the key role in regulating CO_2, while the kidneys serve as the long-term regulators of H^+ and HCO_3^-. Thus, these 2 organ systems are paramount in our discussion of acid-base regulation.

FORMULATING A DIAGNOSIS

Acid-base disturbances can be diagnosed from arterial blood gases (ABGs) using a 3-question method. Given that arterial blood is the source for the diagnostic data, one is actually determining an acidemia or alkalemia. However,

an acidemia or alkalemia is typically indicative of an underlying acidosis or alkalosis, respectively.

An overview of this approach is provided here to lay the framework for remainder of the chapter.

Three-Question Method

Question 1: What is the osis?

- If pH <7.35, then acidosis
- If pH >7.45, then alkalosis

The normal value of pH is 7.4, with the normal range 7.35–7.45, thus the basis of the numbers above. However, one can in fact have an underlying acid-base disorder even though pH is in the normal range.

Question 2: What is the cause of the osis?

To answer this, one looks at bicarbonate next. In the section below, we will go into more detail.

Question 3: Was there compensation?

A calculation must be performed to answer this final question, and this will be covered in detail below. However, bear in mind the following:

- For respiratory disturbances, the kidneys alter total bicarbonate; whether or not compensation has occurred is based upon the patient's measured bicarbonate versus a calculated value of bicarbonate.
- The respiratory system responds quickly and it is important to determine if it has responded appropriately; respiratory compensation compares the patient's measured PCO_2 versus a calculated (predicted) value.

The 4 Primary Disturbances

There are 4 primary acid-base disturbances, each of which results in an altered concentration of H^+. The basic deviations from normal can be an acidosis (excess H^+) or an alkalosis (deficiency of H^+), either of which may be caused by a respiratory or metabolic problem.

- **Respiratory acidosis**: too much CO_2
- **Metabolic acidosis**: addition of H^+ (not of CO_2 origin) and/or loss of bicarbonate from the body
- **Respiratory alkalosis**: not enough CO_2
- **Metabolic alkalosis**: loss of H^+ (not of CO_2 origin) and/or addition of base to the body

Normal systemic arterial values are as follows:

$$pH = 7.4$$

$$HCO_3^- = 24 \text{ mEq/L}$$

$$PCO_2 = 40 \text{ mm Hg}$$

Follow the Bicarbonate Trail

Question 2 asks for the cause of the osis. To answer this, look at the bicarbonate concentration and remember the basic CO_2–bicarbonate reaction, applying mass action. The table below shows the 4 primary disturbances with the resultant bicarbonate changes.

$$CO_2 \leftrightarrow H^+ + HCO_3^-$$

Table VI-5-1. Acute Changes in pH/HCO_3^-

	pH	HCO_3^-
Respiratory acidosis	↓	↑
Metabolic acidosis	↓	↓↓
Respiratory alkalosis	↑	↓
Metabolic alkalosis	↑	↑↑

Respiratory acidosis is characterized by too much CO_2.

- Increasing CO_2 drives the reaction to the right, thereby increasing HCO_3^-.
  - For every 1 mm Hg rise in $PaCO_2$, there is a 0.1 mEq/L increase in HCO_3^-, as a result of the chemical reaction.
  - Thus, there is a **1 : 0.1 ratio** of CO_2 increase to HCO_3^- increase for an **acute (uncompensated) respiratory acidosis.**

Metabolic acidosis causes a marked decrease in HCO_3^- because the addition of H^+ consumes bicarbonate (drives reaction to the left).

- Alternatively, the acidosis could be caused by loss of base (HCO_3^-).

Respiratory alkalosis is characterized by a reduced CO_2.

- Decreasing CO_2 drives the reaction to the left, thereby reducing HCO_3^-.
  - For every 1 mm Hg fall in $PaCO_2$, there is a 0.2 mEq/L decrease in HCO_3^- as a result of the chemical reaction.
  - Thus, there is a **1 : 0.2 ratio** of CO_2 decrease to HCO_3^- decrease for an **acute (uncompensated) respiratory alkalosis.**

Metabolic alkalosis causes a rise in HCO_3^- because the loss of H^+ drives the reaction to the right.

- Alternatively, an alkalosis can be caused by addition of base (bicarbonate) to the body.

Note

One can use pH instead of bicarbonate to determine if a respiratory disturbance is acute or chronic; this is provided at the end-of-chapter Supplemental Information.

COMPENSATION

Respiratory Acidosis

The kidneys compensate by increasing HCO_3^- and eliminating H^+, but the kidneys take days to fully compensate.

- For every 1 mm Hg increase in $PaCO_2$, HCO_3^- increases 0.35 mEq/L as a result of kidney compensation. Thus, there is a **1:0.35 ratio of CO_2 increase to HCO_3^- increase in a chronic (compensated) respiratory acidosis.**

Metabolic Acidosis

Metabolic acidosis is characterized by low pH and HCO_3^-. The drop in pH stimulates ventilation via peripheral chemoreceptors, thus the respiratory system provides the first, rapid compensatory response.

- Winter's equation is used to determine if the respiratory response is adequate.

$$\text{Predicted } PaCO_2 = (1.5 \times HCO_3^-) + 8$$

- The patient's $PaCO_2$ should be within 2(±) of this predicted value, and if so, then respiratory compensation has occurred.

Respiratory Alkalosis

The kidneys compensate by eliminating HCO_3^- and conserving H^+, but the kidneys take days to fully compensate.

- For every 1 mm Hg drop in $PaCO_2$, HCO_3^- decreases 0.5 mEq/L as a result of kidney compensation. Thus, there is a **1:0.5 ratio** of CO_2 decrease to HCO_3^- decrease **in a chronic (compensated) respiratory alkalosis**.
- The maximum low for HCO_3^- is 15 mEq/L.

Metabolic Alkalosis

Similar to a metabolic acidosis, the respiratory system is the first-line compensatory mechanism.

Ventilation decreases to retain CO_2.

- The following equation is used to determine if compensation occurred. It computes the $PaCO_2$, which denotes appropriate compensation.

$$\text{Expected } PaCO_2 = (0.7 \times \text{rise in } HCO_3^-) + 40$$

- The patient's $PaCO_2$ should be within 2(±) of the computed value, but should not exceed 55 mm Hg. The 40 represents the normal $PaCO_2$ (see above).

Additional Important Points

- The body never overcompensates.
  - If it appears that a patient "overcompensated" for a primary disorder, there is likely a second disorder.
- If CO_2 and HCO_3^- go in opposite directions, there is a combined disturbance—either a combined (mixed) respiratory and metabolic acidosis or a combined (mixed) respiratory and metabolic alkalosis.
- Although the opposite direction rule is true, do not presume that it is **required** for someone to have a combined disturbance, i.e., a combined disturbance can still exist even if CO_2 and HCO_3^- go in the same direction.
- Too much CO_2 is a respiratory acidosis.
- Too little CO_2 is a respiratory alkalosis.

PLASMA ANION GAP (PAG)

The total cation charges in the plasma always equal the total anion charges present. However, only major ions are typically measured in a blood sample and an "anion gap" can be determined.

Cations are estimated as the plasma concentration of the major cation, Na^+. It is not usually the case but some clinicians also include K^+ (normal K^+ is 4 mEq/L and if included, then adjust the normal gap accordingly, i.e., add 4).

Anions are estimated as the plasma Cl^- and HCO_3^-.

$$PAG = Na^+ - (Cl^- + HCO_3^-)$$

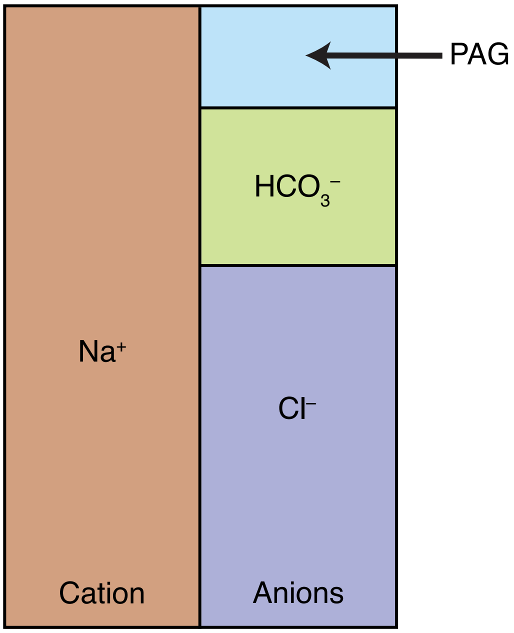


Figure VI-5-2. PAG

Normal values:

Na^+: 140 mEq/L

Cl^-: 104 mEq/L

HCO_3^-: 24 mEq/L

PAG: $Na^+ - (Cl^- + HCO_3^-)$

PAG: 12 ± 2

The anion gap is useful in differentiating the cause of a metabolic acidosis. In most cases, the anion gap increases when the underlying cause involves an organic acid (unmeasured charge is conjugate base of the acid). When the acidosis is the result of bicarbonate loss, e.g., diarrhea, then chloride typically increases resulting in no change in the anion gap.

The more common causes of an elevated and non-elevated gap can be remembered using the mnemonics provided below.

Use the following pneumonics to remember **elevated** and **non-elevated gap metabolic acidoses**:

MUDPILES (elevated gap)	HARDUP (non-elevated gap)
M: Methanol	**H:** Hyperchloremia (parenteral nutrition)
U: Uremia (kidney failure)	**A:** Acetazolamide
D: Diabetic ketoacidosis	**R:** Renal tubular acidosis
P: Paraldehyde	**D:** Diarrhea
I: Iron; Isoniazid	**U:** Ureteral diversion
L: Lactic acidosis	**P:** Pancreatic fistula
E: Ethylene glycol; ethanol ketoacidosis	
S: Salicylates; starvation ketoacidosis; sepsis	

DIAGNOSIS

Now that we have discussed how the basic reaction responds to respiratory and metabolic disturbances and how the body compensates, let's use the 3 questions to quickly determine acid-base abnormalities.

Question 1: What is the osis?

As indicated above, look at pH.

- If pH <7.35, then it's acidosis.
- If pH >7.45, then it's alkalosis.

Question 2: What is the cause of the osis?

Follow the bicarbonate trail.

- If the answer to question 1 is acidosis and HCO_3^- is elevated, then **respiratory acidosis.**
- If the answer to question 1 is acidosis and HCO_3^- is low, then **metabolic acidosis.**
- If the answer to question 1 is alkalosis and HCO_3^- is low, then **respiratory alkalosis.**
- If the answer to question 1 is alkalosis and HCO_3^- is elevated, then **metabolic alkalosis.**

Question 3: Was there compensation?

Respiratory acidosis

Because kidney compensation is slow, it is important to distinguish between **acute (uncompensated)** and **chronic (compensated)** respiratory disturbances.

- If **acute**, there is a 0.1 mEq/L rise in HCO_3^- for every 1 mm Hg increase in $PaCO_2$ (**1:0.1 ratio**).
- If **chronic**, there is a 0.35 mEq/L rise in HCO_3^- for every 1 mm Hg rise in $PaCO_2$ (**1:0.35 ratio**).

For example, a patient has a respiratory acidosis (determined by steps 1 and 2) with $PaCO_2$ 60 mm Hg, which is **20 mm Hg greater than** the normal of 40 mm Hg.

If acute, then bicarbonate will be ~26 ($20 \times 0.1 = 2$; $24 + 2 = 26$). If chronic, then bicarbonate will be ~31 ($20 \times 0.35 = 24 + 7 = 31$).

Metabolic acidosis

The patient's $PaCO_2$ should fall to a level that is ±2 mm Hg of the value computed by Winter's equation:

$$\mathbf{PaCO_2 = (1.5 \times HCO_3^-) + 8}$$

- If the patient's $PaCO_2$ is within 2, then the patient has **metabolic acidosis with respiratory compensation.**
- If it is higher than 2, then the respiratory response is inadequate and the patient has **metabolic and respiratory acidosis.**
- If the patient's $PaCO_2$ is too low, then the patient has a **metabolic acidosis with a respiratory alkalosis.**

For example, a patient has a metabolic acidosis with a HCO_3^- of 10 mEq/L and a $PaCO_2$ of 23 mm Hg. Expected $PaCO_2$ is $(1.5 \times 10) + 8 = 23$ mm Hg, which is what the patient has, thus respiratory compensation is adequate.

Respiratory alkalosis

Again, it is important to distinguish between **acute (uncompensated)** and **chronic (compensated)** respiratory disturbances.

- If **acute,** there is a 0.2 mEq/L fall in HCO_3^- for every 1 mm Hg decrease in $PaCO_2$ (**1:0.2 ratio**).
- If **chronic**, there is a 0.5 mEq/L fall in HCO_3^- for every 1 mm Hg decrease in $PaCO_2$ (**1:0.5 ratio**).

For example, a patient has a respiratory alkalosis (determined by steps 1 and 2) with a $PaCO_2$ of 25 mm Hg, which is **15 mm Hg less than** the normal of 40 mm Hg. If acute, then bicarbonate will be about 21 ($15 \times 0.2 = 3$; $24 – 3 = 21$), but if chronic it will be around 16 ($15 \times 0.5 = 7.5$; $24 – 7.5 = 16.5$).

Note

Remember to calculate the anion gap to differentiate the possible causes of a metabolic acidosis.

Metabolic alkalosis

The patient's $PaCO_2$ should rise to a level that is ±2 the value computed by the following equation (not to exceed 55 mm Hg):

$$\textbf{Expected } PaCO_2 = (0.7 \times \text{rise in } HCO_3^-) + 40$$

- If the patient's $PaCO_2$ is within 2, the patient has **metabolic alkalosis with respiratory compensation.**
- If it is higher than 2, then the patient has **metabolic alkalosis and respiratory acidosis.**
- If the patient's $PaCO_2$ is too low, then the patient has a **metabolic and respiratory alkalosis.**

For example, a patient has a metabolic alkalosis with HCO_3^- 34 mEq/L (**10 greater than normal**) and $PaCO_2$ 47 mm Hg. Expected $PaCO_2$ is $(10 \times 0.7) + 40 = 47$ mm Hg, which is what the patient has, thus respiratory compensation is adequate.

The figure below can be helpful to remember the 3 basic steps for analyzing an ABG and come to the correct diagnosis.

Note

- $Paco_2$ is **increased in respiratory acidosis**; compensatory increase in metabolic alkalosis (formula computes compensation value of $Paco_2$)
- $Paco_2$ is **decreased in respiratory alkalosis**; compensatory decrease in metabolic acidosis (Winter's determines compensation value of $Paco_2$)

Note

Compute anion gap:
[N^a+ – (Cl$^-$ + bicarb)]

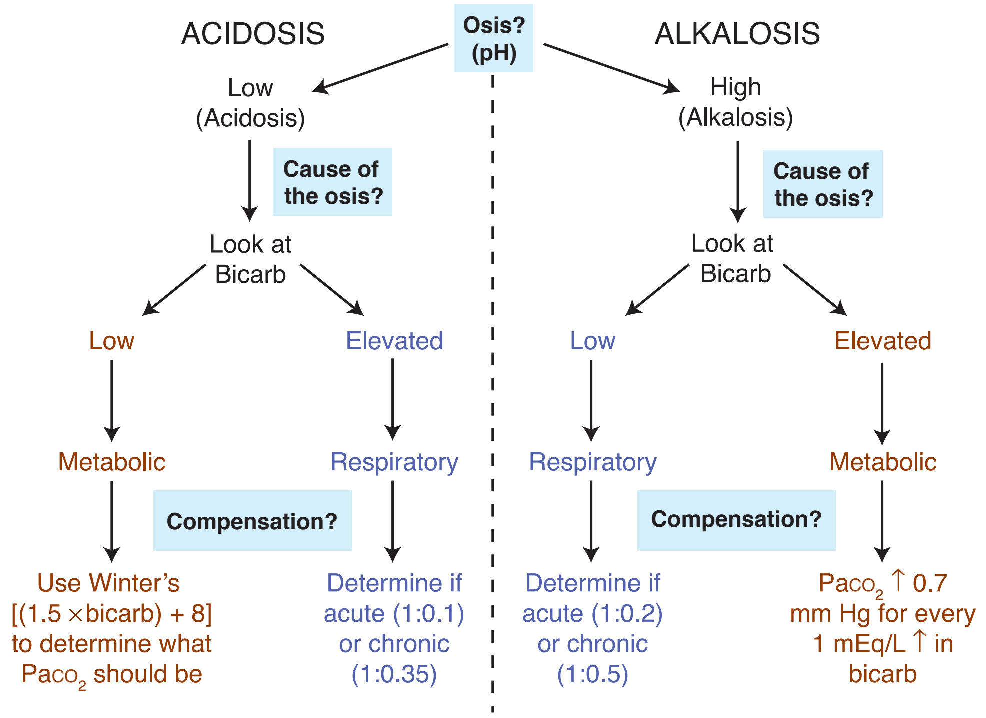


Figure VI-5-3. Analyzing an ABG

Some ABGs showing disturbance follow below:

Example 1: pH 7.3, HCO_3^- 14 mEq/L, PCO_2 30 mm Hg, PO_2 95 mm Hg

Example 2: pH 7.6, HCO_3^- 20 mEq/L, PCO_2 20 mm Hg, PO_2 95 mm Hg

Example 3: pH 7.2, HCO_3^- 30 mEq/L, PCO_2 80 mm Hg, PO_2 70 mm Hg

Example 4: pH 7.6, HCO_3^- 44 mEq/L, PCO_2 52 mm Hg, PO_2 70 mm Hg

Example 5: HCO_3- 20 mEq/L, PCO_2 55 mm Hg

Answers

Example 1: What is the osis? pH is low, so acidosis. **Cause of the osis?** HCO_3^- is low, so metabolic acidosis. **Compensation?** Use Winter's to compute predicted PCO_2: $(14 \times 1.5) + 8 = 29$. Patient's is 30, which is within 2, thus this is a **metabolic acidosis with respiratory compensation.**

Example 2: What is the osis? pH is high, so alkalosis. **Cause of the osis?** HCO_3^- is low, so respiratory alkalosis. **Compensation?** Must determine if acute (uncompensated) or chronic (compensated). PCO_2 is 20 below normal, thus acute: $20 \times 0.2 = 4$, so HCO_3^- will be around 20 (24 – 4). If chronic, it would be $20 \times 0.5 = 10$, so HCO_3^- would be around 14 (24 – 10). The measured equals the predicted acute, thus this is an **acute respiratory alkalosis.**

Example 3: What is the osis? pH is low, so acidosis. **Cause of the osis?** HCO_3^- is high, so respiratory acidosis. **Compensation?** Must determine if acute (uncompensated) or chronic (compensated). PCO_2 is 40 greater than normal, thus acute: $40 \times 0.1 = 4$, so HCO_3^- will be around 28 (24 + 4). If chronic, it will be $40 \times 0.35 = 14$, so HCO_3^- will be around 38 (24 + 14). The measured is much closer to the predicted acute, thus this is an **acute respiratory acidosis.**

Example 4: What is the osis? pH is high, so alkalosis. **Cause of the osis?** HCO_3^- is high, so metabolic alkalosis. **Compensation?** The respiratory compensation is to reduce ventilation, thereby increasing $PaCO_2$. Thus, we need to compute what $PaCO_2$ should be in a patient with this acid-base disorder. Calculation: HCO_3^- is 20 greater than normal of 24, $20 \times 0.7 = 14$, thus $PaCO_2$ should be 14 mm Hg greater than the normal of 40, thus $40 + 14 = 54$ (predicted $PaCO_2$). Patient's is 52, which is within 2, so this is a **metabolic alkalosis with respiratory compensation.**

Example 5: No pH is given, but we can still figure out the acid-base disorder. HCO_3^- is low, so this must be a metabolic acidosis or respiratory alkalosis (review Follow the Bicarbonate Trail). $PaCO_2$ is well above normal, ruling out a respiratory alkalosis, thus there is a **mixed metabolic and respiratory acidosis.** Note that $PaCO_2$ and HCO_3^- went in opposite directions, so we know there is a mixed disturbance. The low HCO_3^- indicates the metabolic acidosis, while the high $PaCO_2$ indicates respiratory acidosis. If this were simply a respiratory acidosis, HCO_3^- would be elevated, not reduced.

GRAPHICAL REPRESENTATION

The Davenport plot (Figure VI-5-4, panels A–D) provides a graphical representation of the preceding discussion. The pH is on the X-axis, and HCO_3^- is on the Y-axis.

At pH 7.4 and HCO_3^- 24, $PaCO_2$ is 40 (panel A). These represent the normal values indicated above. Knowing either of the 2 measurements allows one to calculate the third, using the Henderson-Hasselbalch equation.

$$pH = pK + \log \frac{[HCO_3^-]}{aPCO_2} \qquad pK = 6.10 \qquad [HCO_3^-] \text{ in mmol/L}$$

$$a = 0.0301 \qquad PCO_2 \text{ in mm Hg}$$

Let's now walk through the 3 questions and see the corresponding values on the Davenport plot.

Question 1: What is the osis?

Splitting the graph at pH 7.4, anything to the left represents an acidosis, while anything to the right represents an alkalosis (panel B)

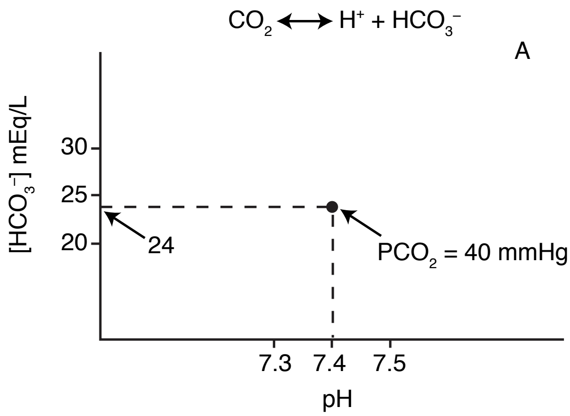


Figure VI-5-4a

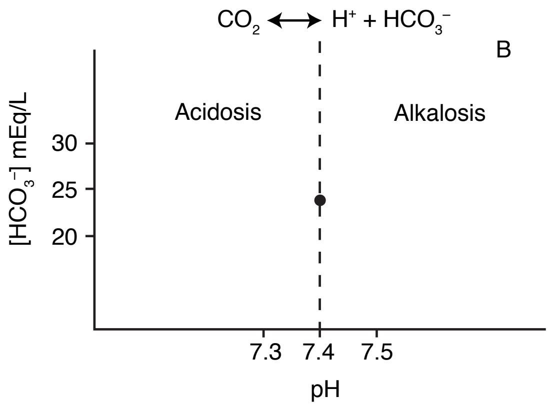


Figure VI-5-4b

Question 2: What is the cause of the osis?

- Respiratory acidosis is characterized by a decrease in pH with concomitant rise in HCO_3^-, while respiratory alkalosis is characterized by a rise in pH with concomitant fall in HCO_3^- (red line in panel C)
- Metabolic acidosis is a decrease in pH and HCO_3^-, while metabolic alkalosis is an increase in pH and HCO_3^-. This is denoted by the purple line in panel C of the figure. Note that PCO_2 is the same

at all points along the purple line, hence the term *CO_2 isobar*. In other words, the purple line denotes metabolic changes without accompanying CO_2 changes.

- This separates the graph into 4 quadrants (panel C). Points falling within one of these quadrants will have the respective primary acid-base disturbance.
  - Upper left, respiratory acidosis
  - Lower left, metabolic acidosis
  - Lower right, respiratory alkalosis
  - Upper right, metabolic alkalosis

Question 3: Was there compensation?

- There is no need to be as quantitative when using the Davenport plot to answer this question. Simply determine if the pH moved toward normal (7.4).
- The red respiratory and purple metabolic lines represent buffer lines, thus compensation follows the same plane as it moves toward 7.4.
- The direction the curve shifts if compensation occurred is depicted by the solid black arrows (panel D).

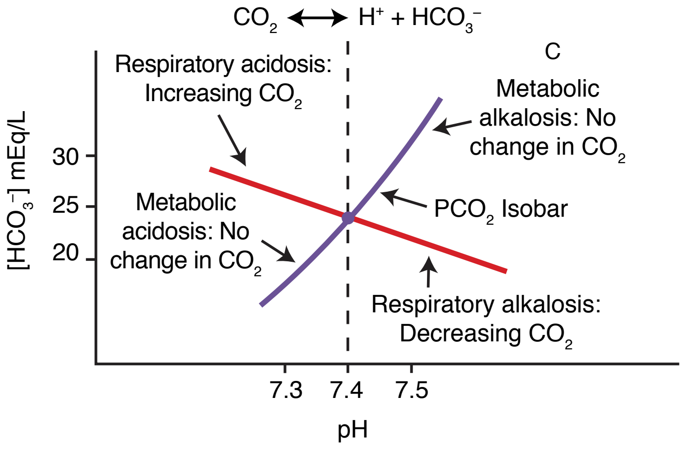


Figure VI-5-4c

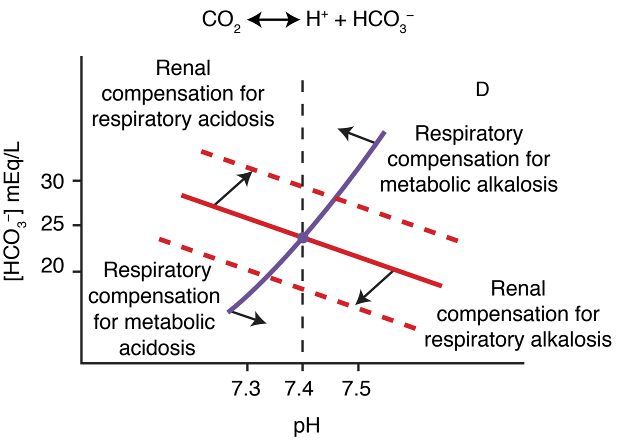


Figure VI-5-4d

The Davenport plot below shows unlabelled points. You are encouraged to try and indicate the correct acid-base disturbance depicted by the points.

Answers are provided below.

A: acute (uncompensated) respiratory acidosis

B: chronic (compensated) respiratory acidosis

C: metabolic alkalosis with respiratory compensation

D: metabolic acidosis with respiratory compensation

E: acute (uncompensated) respiratory alkalosis

F: chronic (compensated) respiratory alkalosis

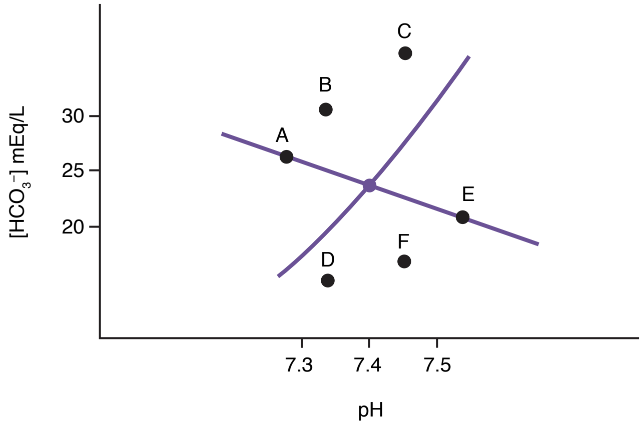


Figure VI-5-5.

SUPPLEMENTAL INFORMATION

Respiratory Acidosis

A respiratory acidosis is the result of CO_2 accumulating in the body, which causes an increase in H^+ (or decrease in pH) and an increase in HCO_3^-. Quantitatively, in the acute (uncompensated) state, for every 10 mm Hg rise in $PaCO_2$, HCO_3^- rises about 1 mEq/L and pH falls by 0.08 pH units.

Respiratory acidosis can be caused by the following:

- Respiratory center depression (anesthetics, morphine)
- Pulmonary edema, cardiac arrest
- Airway obstruction
- Muscle relaxants
- Sleep apnea
- Chronic obstructive lung disease
- Neuromuscular defects (multiple sclerosis, muscular dystrophy)
- Obesity hypoventilation syndrome

Summary: The **cause** is an increase in $PaCO_2$. The **result** is a decrease in pH and slight increase in HCO_3^-.

Metabolic Acidosis

This is caused by a gain in fixed (not of CO_2 origin) acid and/or a loss of base. The increased H^+ drives the reaction to the left, decreasing HCO_3^-. Forcing the reaction to the left produces some CO2, but the hyperventilation evoked by the acidosis eliminates CO_2.

As described above, the possible cause of the acidosis can be narrowed by determining the anion gap (MUDPIILES for elevated; HARDUP if gap is normal).

Summary: The **cause** is a gain in H^+ as fixed acid and/or a loss of HCO_3^- (via GI tract or kidney). The **result** is a decrease in pH and HCO_3^- and compensatory fall in $PaCO_2$.

Respiratory Alkalosis

This is caused by an increase in alveolar ventilation relative to body production of CO_2 (hyperventilation). Quantitatively, in the acute (uncompensated) state, for every 10 mm Hg decrease in $PaCO_2$, HCO_3^- decreases about 2 mEq/L and pH rises 0.08 pH units.

Respiratory alkalosis can be caused by:

- Anxiety
- Fever
- Hypoxemia
- Pneumothorax (in some cases)
- Ventilation–perfusion inequality
- Hypotension
- High altitude

Summary: The **cause** is a decrease in $PaCO_2$. The **result** is a decrease in H^+ (increased pH) and slight decrease in HCO_3^-.

Metabolic Alkalosis

This is caused by a loss of fixed acid and/or gain of base. The decreased H^+ forces the reaction to the right, increasing HCO_3^-.

A compensatory rise in $PaCO_2$ is expected because the alkalosis decreases ventilation.

Causes:

- Vomiting or gastric suctioning
- Loop and thiazide diuretic use
- Bartter, Gitelman, and Liddle syndromes
- Intracellular shift of hydrogen ions as in hypokalemia
- Primary hyperaldosteronism
- Loss of bicarbonate-free fluid (contraction alkalosis)

Summary: The **cause** is a loss of H^+ and/or gain in HCO_3^-. The **result** is an increase in pH and HCO_3^-; compensatory rise in $PaCO_2$.

Recall Question

A 17-year-old woman is found to have type 1 diabetes mellitus. Which of the following metabolic acid-base disorders is most likely to be seen in her?

A. Decreased anion gap metabolic acidosis

B. Respiratory acidosis with metabolic compensation

C. Respiratory alkalosis with metabolic compensation

D. Increased anion gap metabolic acidosis

E. Metabolic alkalosis with respiratory compensation

Answer: D

PART VII

Endocrinology

General Aspects of the Endocrine System

1

Learning Objectives

- ❑ Demonstrate understanding of overview of hormones
- ❑ Answer questions about disorders of the endocrine system

HORMONES

Lipid- Versus Water-Soluble Hormones

There are several major differences between the lipid-soluble hormones and the water-soluble hormones.

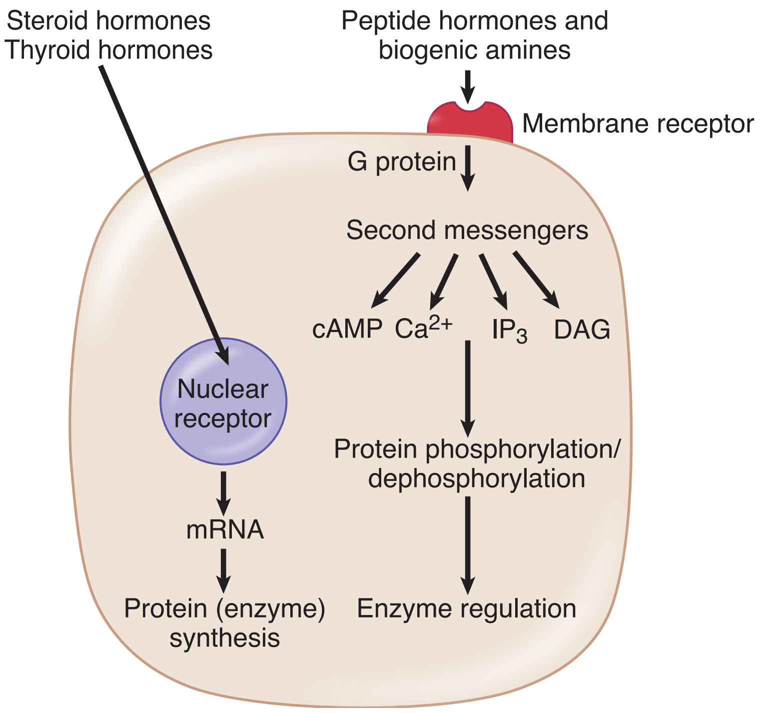
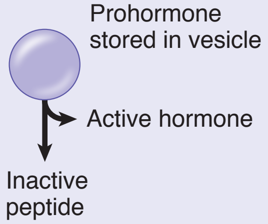


Figure VII-1-1. Signal Transduction Mechanisms

IP_3: inositol triphosphate

DAG: diacylglycerol

Table VII-1-1. Major Classes of Hormones

	Lipid-Soluble Hormones (steroids, thyroid hormones)	Water-Soluble Hormones (peptides, proteins)
Receptors	Inside the cell, usually in nucleus	Outer surface of the cell membrane
Intracellular action	Stimulates synthesis of specific new proteins	• Production of second messengers, e.g., cAMP • Insulin does not utilize cAMP, instead activates membrane-bound tyrosine kinase • Second messengers modify action of intracellular proteins (enzymes)
Storage	• Synthesized as needed • Exception: thyroid hormones	• Stored in vesicles • In some cases, prohormone stored in vesicle along with an enzyme that splits off the active hormone 
Plasma transport	• Attached to proteins that serve as carriers • Exception: adrenal androgens	Dissolved in plasma (free, unbound)
Half-life	Long (hours, days) $\propto$ to affinity for protein carrier	Short (minutes) $\propto$ to molecular weight

Protein-Bound and Free Circulating Hormones

The liver produces proteins that bind lipid-soluble hormones, e.g.:

- cortisol-binding globulin
- thyroid-binding globulin
- sex hormone-binding globulin (SHBG)

Equilibrium

The lipid-soluble hormone circulating in plasma bound to protein is in equilibrium with a small amount of free hormone. It is the free form that is available to the tissues, and thus the free unbound form normally determines the plasma activity. It is the free form that also creates negative feedback.

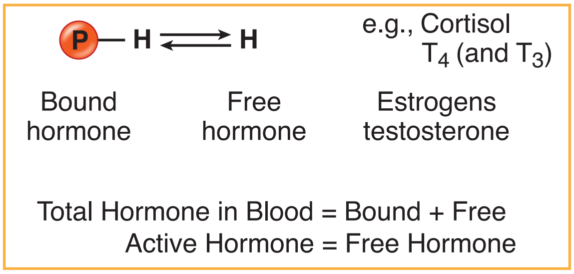


Figure VII-1-2. Transport of Lipid-Soluble Hormones

Role of the liver

If the liver changes its production and release of binding proteins, the circulating level of **bound hormone will change.** However, under most conditions the level of **free hormone will remain constant.**

Modulation

Liver dysfunction and androgens can decrease and estrogens can increase the circulating level of binding proteins. For example, a rise in circulating estrogen causes the release of more binding protein by the liver, which binds more free hormone. The transient decrease in free hormone reduces negative feedback to the hormone-secreting tissue. The increased secretion of free hormone quickly returns the plasma free hormone to normal.

This explains why during pregnancy and other states with a rise in estrogen levels:

- Total plasma lipid-soluble hormone increases.
- Free plasma hormone remains constant at a normal level; thus, the individual does not show signs of hyperfunction.

Hormone Receptors

Hormone specificity

A hormone affects only cells that possess receptors specific to that particular hormone.

For example, both adrenocorticotropic hormone (ACTH) and luteinizing hormone (LH) increase the secretion of steroid hormones. However, ACTH does so only in the adrenal cortex and LH only in gonadal tissue.

Hormone activity

Under normal conditions, receptors are not saturated; that is, extra receptors exist. Therefore:

- Normally, the number of hormone receptors is not rate-limiting for hormone action.
- The plasma concentration of free hormone is usually indicative of activity.

Resistance to hormone action

- Abnormalities in receptors or events distal to the ligand-receptor interaction, often due to chronic elevation of circulating hormone (e.g., type II diabetes) or drug therapy.
- Under these conditions receptors are often saturated.
- Reduction of hormone levels often produces some recovery in sensitivity.
- The clinical presentation is often one of normal or elevated hormone levels but with reduced or absent peripheral manifestations of the hormone and a failure of replacement therapy to correct the problem.

Permissive action

A phenomenon in which one type of hormone must be present before another hormone can act; for example, cortisol must be present for glucagon to carry out gluconeogenesis and prevent hypoglycemia.

Measurement of Hormone Levels

Plasma analysis

- Provides information at the time of sampling only and may not reflect the overall secretion rate
- When hormone secretion is episodic, single sampling may reflect peaks (erroneous hyperfunction) or nadirs (erroneous hypofunction). Pulsatile secretion, diurnal and cyclic variation, age, sleep entrainment, and hormone antagonism must all be considered in evaluating circulating levels.
  - Growth hormone is secreted in pulses and mainly at night. This is not reflected in a fasting morning sample. However, growth hormone stimulates the secretion of IGF-I which circulates attached to protein and has a long half-life (20 hours). Plasma IGF-I measured at any time during the day is usually a good index of overall growth hormone secretion.
  - Thyroid is a fairly constant system and T4 has a half-life of about 6–7 days. Thus, a random measurement of total T4 is usually a good estimate of daily plasma levels.

Urine analysis

- Restricted to the measurement of catecholamines, steroid hormones, and water-soluble hormones such as hCG and LH.
- A distinct advantage of urine analysis is that it provides an integrated sample.
  - A "24-hour urine free cortisol" is often necessary to pick up a low-level Cushing's syndrome and to eliminate the highs and lows of the normal circadian rhythm.

DISORDERS OF THE ENDOCRINE SYSTEM

Primary versus Secondary Disorders

A **primary disorder** means dysfunction originating in the endocrine gland itself, either hyper- or hypofunction. Examples include:

- Excess cortisol from an adrenal adenoma (Cushing syndrome)
- Decreased thyroid secretion (Hashimoto's thyroiditis)
- Reduced ADH secretion (central diabetes insipidus)

A **secondary disorder** indicates that a disturbance has occurred causing the gland to secrete more or less of the hormone. Examples include:

- Cushing disease (pituitary adenoma secreting ACTH) resulting in hypercortisolism
- A dehydrated patient with elevated plasma osmolality causing high ADH level

Hypofunction

Hypofunction is caused by autoimmune disease (e.g., type I diabetes, hypothyroidism, primary adrenal insufficiency, gonadal failure), tumors, hemorrhage, infection, damage by neoplasms

Evaluation is with a stimulation test:

- Hypothalamic hormones test anterior pituitary reserve
- Injection of the pituitary trophic hormone (e.g., ACTH) tests target gland reserve.
- Failure of growth hormone release after arginine injection

Hyperfunction

Hyperfunction is caused by hormone-secreting tumors, hyperplasia, autoimmune stimulation, ectopically produced peptide hormones (e.g., ACTH, ADH)

Evaluation is with a suppression test:

- Failure of glucose to suppress growth hormone diagnostic for acromegaly
- Failure of dexamethasone (low dose) to suppress cortisol diagnostic for hypercortisolism
- Multiple endocrine neoplasia (MEN) represents a group of inheritable syndromes characterized by multiple benign or malignant tumors.
    - **MEN 1**: hyperparathyroidism, endocrine pancreas, and pituitary adenomas
    - **MEN 2A**: medullary carcinoma of the thyroid, pheochromocytoma, hyperparathyroidism
    - **MEN 2B**: medullary carcinoma of the thyroid, pheochromocytoma, mucosal neuromas (tongue and lips), GI disorders, thick eyelids, and marfanoid habitus (muscle, joint, spinal, bone alterations)

Gland Structure and Size

When an endocrine gland does not receive its normal stimulus, it generally undergoes a reversible atrophy.

- Long-term high doses of glucocorticoids suppress the ACTH-adrenal axis. Withdrawal of therapy can require up to a year for complete recovery.
- Overstimulation of endocrine tissue can cause cell proliferation or hypertrophy in addition to hormone overproduction.
  - In Graves' disease, overstimulation of the thyroid tissues causes cell proliferation and this polyclonal expansion creates a goiter in addition to hyperthyroidism.
- Tumors, which are generally monoclonal expansions, may also create a hyperfunction. Others produce little if any hormone but are still disease-producing because of the compressive (mass) effect of the additional tissue.

Hypothalamic–Anterior Pituitary System

2

Learning Objectives

- ❑ Solve problems concerning hypothalamic–anterior pituitary axis
- ❑ Solve problems concerning disorders of the hypothalamic-anterior pituitary axis

HYPOTHALAMIC–ANTERIOR PITUITARY AXIS

- The hormones in this system are all water-soluble.
- The hypothalamic hormones are synthesized in the neuron cell body, packaged in vesicles, and transported down the axon to be stored and released from the nerve terminals.
- Pituitary is located in the bony sella turcica at the base of the skull. It hangs from the hypothalamus by a stalk (the infundibulum) and is controlled by the hypothalamus. The dura membrane (diaphragm sellae) separates it from and prevents cerebrospinal fluid from entering the sella turcica.
- Optic chiasm is 5–10 mm above this diaphragm.
- In the hypothalamic–anterior pituitary system, hormonal release is mainly pulsatile. A possible exception is the thyroid system.
- The pulsatile release of GnRH prevents downregulation of its receptors on the gonadotrophs of the anterior pituitary. A constant infusion of GnRH will cause a decrease in the release of both LH and FSH.

The hypothalamic–anterior pituitary system is summarized below.

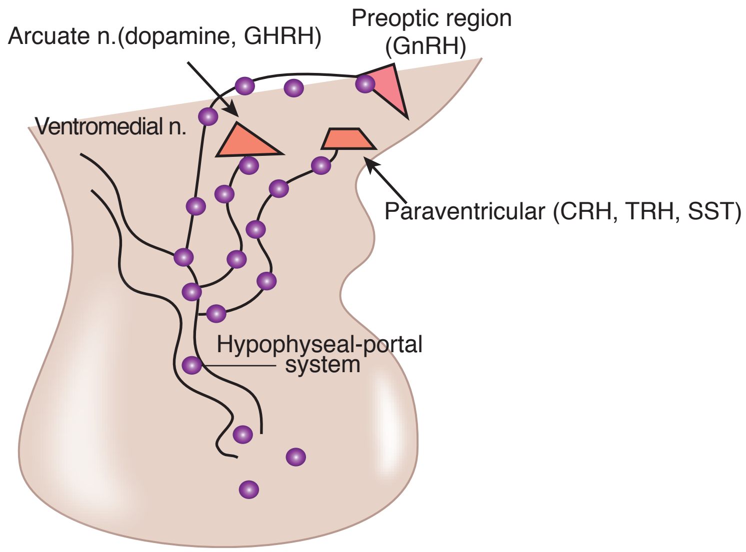


Figure VII-2-1. Hypothalamic–Anterior Pituitary Axis

- The hypothalamic hormones, thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone (CRH), growth hormone–releasing hormone (GHRH), somatostatin (SST), and dopamine are synthesized in neuronal cell bodies in the arcuate and paraventricular nuclei; gonadotropin-releasing hormone (GnRH) is synthesized in the preoptic nucleus.
- The nerve endings all come together in the median eminence region of the hypothalamus. The hormones are then secreted into the hypophyseal-portal system and transported to the anterior pituitary.
- Hypothalamic hormones bind to receptors on cells of the anterior pituitary and modify the secretion of thyroid-stimulating hormone (TSH) (thyrotropin), corticotropin (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and prolactin.

Effect of Each Hypothalamic Hormone on Anterior Pituitary

+ = releaser
– = inhibitor

Hypothalamus		Pituitary Target	Secretion
TRH	+ →	Thyrotrophs (10%)	TSH
CRH	+ →	Corticotrophs (10–25%)	ACTH
GnRH*	+ →	Gonadotrophs (10–15%)	LH, FSH
GHRH**	+ →	Somatotrophs (50%)	GH
SST	– →		
Dopamine***	– →	Lactotrophs (10–15%)	Prolactin
TRH (elevated)	+ →		

*High frequency pulses favor LH, low frequency pulses favor FSH

**The fact that eliminating hypothalamic input causes a decrease in growth hormone secretion indicates that GHRH is the main controlling factor.

***When the connection between the hypothalamus and the anterior pituitary is severed (e.g., there is damage to the pituitary stalk), secretion of all anterior pituitary hormones decreases, except prolactin, which increases. The secretion of prolactin increases because a chronic source of inhibition (dopamine) has been removed.

Figure VII-2-2. Control of the Anterior Pituitary

TRH: thyrotropin-releasing hormone
TSH: thyroid-stimulating hormone or thyrotropin
CRH: corticotropin-releasing hormone
ACTH: adrenocorticotropic hormone or corticotropin
GnRH: gonadotropin-releasing hormone
LH: luteinizing hormone
FSH: follicle-stimulating hormone
GHRH: growth hormone–releasing hormone
GH: growth hormone
SST: somatostatin

DISORDERS OF THE HYPOTHALAMIC–ANTERIOR PITUITARY AXIS

Hypopituitarism

Hypopituitarism can be inherited but other causes include head trauma (most common), mass effects of tumors, inflammation, or vascular damage

- Characteristic sequential loss of function: growth hormone and gonadotropin, followed by TSH then ACTH and finally prolactin.
- Isolated deficiency:

  Growth hormone: sporadic or familial

  Gonadotropins: Kallman syndrome – (tertiary) defective

  GnRH synthesis; ↓ LH ↓ FSH ↓ sex steroids

  ACTH, TSH, and prolactin extremely rare deficiencies, usually a sign of panhypopituitarism
- Craniopharyngioma is the most common tumor affecting the hypothalamic–pituitary system in children (pituitary adenomas rare).
- Although one would predict the trophic hormones to be low, this is not often the case. Typically, they are in the normal range, but their level is inadequate to stimulate peripheral glands adequately.
- From an academic perspective, stimulation tests include: GnRH → LH, FSH; TRH → TSH, prolactin; insulin infusion → GH, ACTH

Sheehan syndrome

The pituitary in pregnancy is enlarged and therefore more vulnerable to infarction. Sometimes when delivery is associated with severe blood loss, the ensuing shock causes arteriolar spasm in the pituitary with subsequent ischemic necrosis. Some degree of hypopituitarism has been reported in 32% of women with severe postpartum hemorrhage. Symptoms vary, depending on the extent and location of pituitary damage, but failure to lactate for days following birth is a strong sign of pituitary damage.

Pituitary Adenomas

Pituitary adenomas are the most common cause of hypothalamic–pituitary dysfunction.

- Microadenomas (< 1 cm diameter) are characterized by hormonal excess, no panhypopituitarism, treatable, e.g., ACTH (Cushing disease)
- Hypogonadism is the most common manifestation
- Usually benign and can autonomously secrete hormone leading to hyperprolactinemia (60%), acromegaly (growth hormone 20%), and Cushing disease (ACTH 10%)
- Prolactinomas associated with hypogonadism and galactorrhea
- MEN 1 association
- Macroadenomas (>1 cm diameter): mass effect, larger tumors with suprasellar extension, associated with panhypopituitarism and visual loss

Recall Question

A 48-year-old woman undergoes surgical removal of her pituitary macroadenoma when the pituitary stalk is accidentally cauterized. An increase in which of the following is most likely to be noted on labs?

A. Thyroid stimulating hormone

B. Prolactin

C. Cortisol

D. Dopamine

E. Follicle stimulating hormone

Answer: B

Posterior Pituitary 3

Learning Objectives

- ❑ Answer questions about hormones of the posterior pituitary
- ❑ Explain information related to regulation of ECF volume and osmolarity
- ❑ Answer questions about pathophysiologic changes in ADH secretion
- ❑ Use knowledge of hyponatremia

HORMONES OF THE POSTERIOR PITUITARY

- Made up of distal neuron terminals
- Secreted hormones; arginine vasopressin (ADH), oxytocin (see chapter 11)—both are peptide hormones.
- Cell bodies located in the supraoptic nucleus and paraventricular nucleus of the hypothalamus.
- ADH is a major controller of water excretion and regulator of extracellular osmolarity.
- The osmoreceptor neurons in the hypothalamus are extremely sensitive and are able to maintain ECF osmolarity within a very narrow range.
- There is a downward shift in plasma osmolarity regulation in pregnancy, the menstrual cycle, and with volume depletion. In the latter case osmoregulation is secondary to volume regulation; a return of circulating volume occurs even though osmolarity decreases.
- Volume receptors are less sensitive than osmoreceptors and a change of 10–15% in volume is required to produce a measurable change in ADH.
- Angiotensin II and CRH can stimulate the release of ADH.

Figure VII-3-1 illustrates the neural control mechanisms that regulate secretion of ADH by the posterior pituitary. The principal inputs are inhibition by baroreceptor and cardiopulmonary mechanoreceptors (see Part IV, Chapter 3) and stimulation by osmoreceptors.

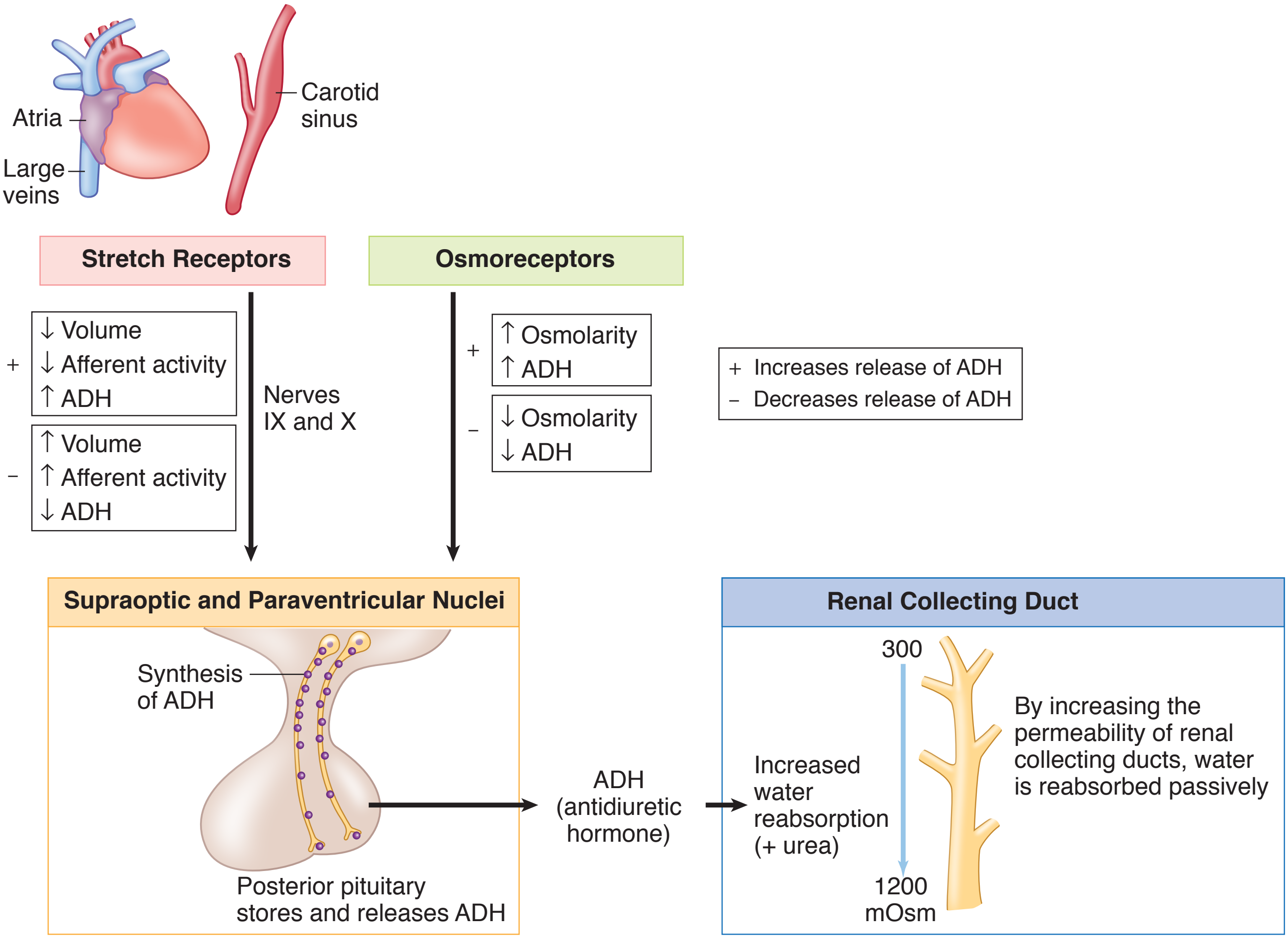


Figure VII-3-1. Neural Control Mechanism

Synthesis and Release of ADH

ADH is synthesized in the supraoptic (SO) and paraventricular (PVN) nuclei of the hypothalamus; it is stored and released from the posterior pituitary.

- Osmoreceptors are neurons that respond to increased plasma osmolarity, principally plasma sodium concentration. They synapse with neurons of the SO and PVN and stimulate them to secrete ADH from the posterior pituitary. They also stimulate consumption of water through hypothalamic centers that regulate thirst.
- The SO and PVN also receive input from cardiopulmonary mechanoreceptors, as well as arterial baroreceptors. High blood volume or blood pressure tends to inhibit secretion of ADH.
- Secretion of ADH is most sensitive to plasma osmolarity (1%); however, if blood volume decreases by 10% (such as hemorrhage) or cardiac output falls, high levels of ADH are secreted even if it causes abnormal plasma osmolarity.

Note

ADH is also stimulated by Ang II and CRH.

Action of ADH

The main target tissue is the renal collecting duct (V2 receptors).

- ADH increases the permeability of the duct to water by placing water channels (aquaporins) in the luminal membrane.
- ADH, acting via the V1 receptor, contracts vascular smooth muscle.

REGULATION OF ECF VOLUME AND OSMOLARITY

Osmoregulation

- An increase of only 1% in the osmolality of the ECF bathing the hypothalamic osmoreceptors evokes an increase in ADH secretion.
- A similarly sized decrease in osmolality decreases ADH secretion.
- In this manner, ECF osmolality is kept very close to 285 mOsm/Kg.

Volume Regulation

- Stimuli arising from stretch receptors act to chronically inhibit ADH secretion.
- Decreases in blood volume cause venous and arterial stretch receptors to send fewer signals to the CNS, decreasing chronic inhibition of ADH secretion.
- This mechanism is especially important for restoring ECF volume following a hemorrhage.

Effect of Alcohol and Weightlessness on ADH Secretion

Ingesting ethyl alcohol or being in a weightless environment suppresses ADH secretion. In weightlessness, there is a net shift of blood from the limbs to the abdomen and chest. This results in greater stretch of the volume receptors in the large veins and atria, thus suppressing ADH secretion. Water immersion to the neck produces a similar phenomenon.

Clinical Correlate

Circulating levels of BNP correlate well with the degree of dilation in heart failure. For this reason, the plasma level of BNP is sometimes used as a marker for the severity of heart failure.

Bridge to Pharmacology

Sacubitril is a drug that inhibits neprilysin. By inhibiting neprilysin, ANP and BNP levels remain elevated for longer periods of time, leading to natriuresis, vasodilation, and reduced cardiac fibrosis and hypertrophy.

Sacubitril is used in combination with the ARB valsartan because blocking neprilysin also increases angiotensin II, and valsartan helps to counteract the deleterious effects of high levels of angiotensin II. Although ANP/BNP can inhibit renin (and thus angiotensin II), in the setting of dilated hearts, their ability to inhibit renin is reduced and/or absent (precise mechanism is unknown).

Side effects of sacubitril/valsartan are angioedema and cough (as indicated above, neprilysin breaks down bradykinin). This combination has been shown to improve mortality in patients with systolic heart failure compared to ACE inhibitors alone.

Natriuretic Peptides

ANP is the hormone secreted by the heart. It is found throughout the heart but mainly in the right atrium. The stimuli which release ANP (two peptides are released) are:

- Stretch, an action independent of nervous involvement
- CHF and all fluid overload states

ANP increases sodium loss (natriuresis) and water loss by the kidney because of, in part, an increase in glomerular filtration rate due to:

- ANP-mediated dilation of the afferent arteriole
- ANP-mediated constriction of the efferent arteriole

ANP also increases sodium loss (natriuresis) and water loss (diuresis) by the kidney because it inhibits renin and aldosterone release as well as the reabsorption of sodium and water in the collecting duct.

The physiologic importance of ANP is not known because it has not been possible to identify or produce a specific deficiency state in humans. However, ANP secretion increases in weightlessness and submersion to the neck in water, while renin, aldosterone, and ADH secretion decrease. It may play a role in normal regulation of the ECF osmolality and volume.

ANP tends to antagonize the effects of aldosterone and ADH.

A normal ANP level is used to exclude CHF as a cause of dyspnea.

In addition to ANP, the heart produces brain natriuretic peptide (BNP).

- In the normal heart very little BNP is produced and secreted. BNP has the same effects as ANP, and both are vasoactive peptides.
- However, if the heart dilates, e.g., heart failure, both ANP and BNP secretion are markedly elevated. Studies suggest that they may reduce cardiac fibrosis and hypertrophy in systolic heart failure, and they vasodilate arterioles thereby reducing blood pressure.
- ANP and BNP are metabolized by an enzyme known as neprilysin. However, neprilysin is not selective for these peptides because it also breaks down angiotensin II and bradykinin.

PATHOPHYSIOLOGIC CHANGES IN ADH SECRETION

Diabetes Insipidus

The consequences can be explained on the basis of the lack of an effect of ADH on the renal collecting ducts.

Central diabetes insipidus (CDI)

- Sufficient ADH is not available to affect the renal collecting ducts.
- Causes include familial, tumors (craniopharyngioma), autoimmune, trauma
- Pituitary trauma – transient diabetes insipidus

- Sectioning of pituitary stalk – triphasic response: diabetes insipidus, followed by SIADH, followed by a return of diabetes insipidus
- Destruction of the hypothalamus from any cause can lead to diabetes insipidus. Forms of hypothalamic destruction are stroke, hypoxia, head trauma, infection, cancer or mass lesions.
- CDI = ADH deficiency. CDI is treated with replacing ADH as vasopressin or DDAVP (desmopressin).

Nephrogenic diabetes insipidus

- Due to inability of the kidneys to respond to ADH
- Causes include familial, acquired, drugs (lithium)
- Hypokalemia
- Hypercalcemia

Lithium, low potassium, and high calcium all diminish ADH's effectiveness on principal cells. The precise mechanism is still unclear, but it may involve disruption in the ability to traffic aquaporins to the luminal membrane of principal cells of the kidney.

Table VII-3-1. Differential Diagnosis Following Water Deprivation

	Plasma Osm	Urine Osm	Plasma ADH	Urine Osm Post Desmopressin
Normal	297	814	↑	815
Central DI*	342	102	↓	622
Nephrogenic	327	106	↑	118

* Patients with partial central DI will concentrate their urine somewhat but will achieve an additional boost following desmopressin.

Syndrome of Inappropriate ADH Secretion (SIADH)

Excessive secretion of ADH causes an inappropriate increased reabsorption of water in the renal collecting duct.

Causes

- Ectopic production of ADH (any CNS or small cell lung pathology)
- Drug induced: SSRI, carbamazepine
- Lesions in the pathway of the baroreceptor system

Pathophysiology

- Increased water retention, hyponatremia, but clinically euvolumic
- Inappropriate concentration of urine, often greater than plasma osmolarity
- With hyponatremia, a normal person should have urine sodium and osmolarity that are low. In SIADH, it is a disease because urine sodium and osmolarity are inappropriately high.

Treatment

- Fluid restriction but not salt restriction
- Sodium disorders cause neurological symptoms.
- Only mild hyponatremia from SIADH can be managed with fluid restriction.
- Severe disease needs 3% hypertonic saline or V2 receptor antagonists.
- Conivaptan and tolvaptan are V2 receptor antagonists; they stop ADH effect on kidney tubule.

Summary of Changes

Table VII-3-2. The Effects of Diabetes Insipidus, Dehydration, SIADH, and Primary Polydipsia

	Diabetes Insipidus	Dehydration	SIADH	Primary Polydipsia
1. Permeability of collecting ducts to H_2O	↓	↑	↑	↓
2. Urine flow	↑	↓	↓	↑
3. Urine osmolarity	↓	↑	↑	↓
4. ECF volume	↓	↓	↑	↑
5. ECF osmolarity* (Na concentration)	↑	↑	↓	↓
6. ICF volume	↓	↓	↑	↑
7. ICF osmolarity	↑	↑	↓	↓

* Overt physical and laboratory signs of dehydration do not necessarily develop unless there is a defect in thirst stimulation.

HYPONATREMIA

Hyponatremia is one of the most common disorders of fluid and electrolyte balance in hospitalized patients. It is usually equivalent to a hypo-osmolar state (exception hyperglycemia). It involves both solute depletion and water retention, although water retention is usually the more important factor.

- Solute depletion can occur from any significant loss of ECF fluid. The hyponatremia is the result of replacement by more hypotonic fluids.
- When it develops **rapidly** (<48 hours) and is severe (Na <120 mEq/L), patient is at risk for seizures and respiratory arrest. Often treated aggressively with hypertonic saline (3%) and diuretics or ADH antagonists.
- When it develops more **slowly**, it appears to be well-tolerated and patient is asymptomatic. Aggressive treatment may result in “central pontine myelinolysis.” General recommendation is to slowly raise Na concentration over a period of days.

Subgroups

Hypervolemia

Caused by marked reduction in water excretion and/or increased rate of water ingestion. Would include congestive heart failure and cirrhosis

Hypovolemia

Indicates solute depletion. Would include mineralocorticoid deficiency, diuretic abuse, renal disease, diarrhea, and hemorrhage

Clinical euvolemia

Would include SIADH and primary (psychogenic) polydipsia. A clinically equivalent presentation may occur in glucocorticoid deficiency or hypothyroidism.

Recall Question

ADH secretion is most sensitive to changes in which of the following?

A. Plasma osmolarity

B. Plasma volume

C. Cardiac output

D. Ejection fraction

E. Peripheral vascular resistance

Answer: A

4 Adrenal Cortex

Learning Objectives

- ❑ Use knowledge of functional regions of the adrenal gland
- ❑ Demonstrate understanding of biosynthetic pathways of steroid hormone synthesis
- ❑ Interpret scenarios on physiologic actions of glucocorticoids
- ❑ Solve problems concerning control of adrenocorticotropin and cortisol secretion
- ❑ Demonstrate understanding of physiologic actions of aldosterone
- ❑ Explain information related to control of aldosterone secretion
- ❑ Explain information related to glucocorticoid and mineralocorticoid disorders
- ❑ Explain information related to enzyme deficiencies

FUNCTIONAL REGIONS OF THE ADRENAL GLAND

The figure below summarizes each adrenal region.

- ACTH controls the release of both cortisol and adrenal androgens.
- Aldosterone is stimulated by a rise in angiotensin II and/or K^+.

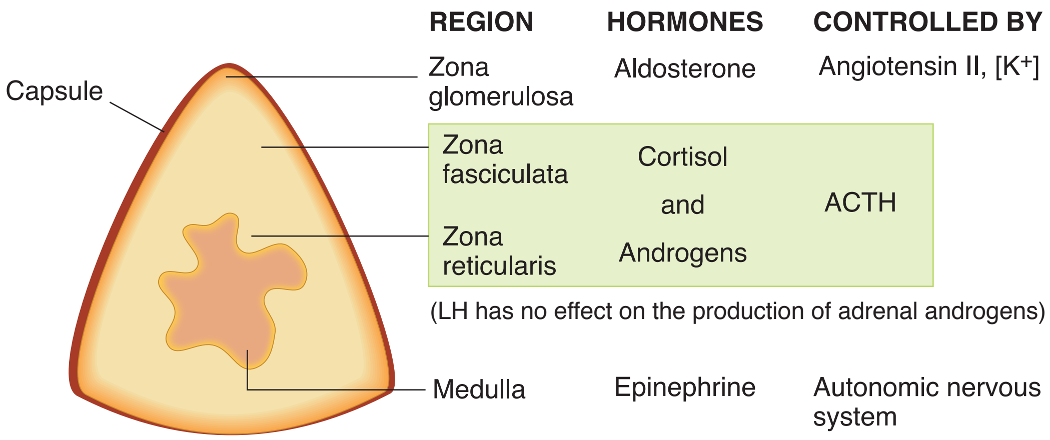


Figure VII-4-1. Adrenal Cortex Regions

Consequences of the Loss of Regional Adrenal Function

Zona glomerulosa: The **absence of the mineralocorticoid, aldosterone**, results in:

- Loss of Na^+
- Decreased volume of the ECF
- Low blood pressure
- Circulatory shock
- Death

Zona fasciculata, zona reticularis: The **absence of the glucocorticoid, cortisol,** contributes to:

- Circulatory failure, because without cortisol, catecholamines do not exert their normal vasoconstrictive action.
- An inability to readily mobilize energy sources (glucose and free fatty acids) from glycogen or fat. Under normal living conditions, this is not life-threatening; however, under stressful situations, severe problems can arise. For example, fasting can result in fatal hypoglycemia.

If problems develop with anterior pituitary secretion, glucocorticoid secretion may be affected, but the mineralocorticoid system remains intact.

BIOSYNTHETIC PATHWAYS OF STEROID HORMONE SYNTHESIS

Synthetic Pathways

The figure below shows a composite of the synthetic pathways in all steroid hormone-producing tissues. A single tissue has only the pathways necessary to produce the hormones normally secreted by that particular tissue. For example, the zona glomerulosa has only the pathways of the first column because the main output of the zona glomerulosa is aldosterone. Cholesterol is pulled off circulating LDL or made de novo by acetate.

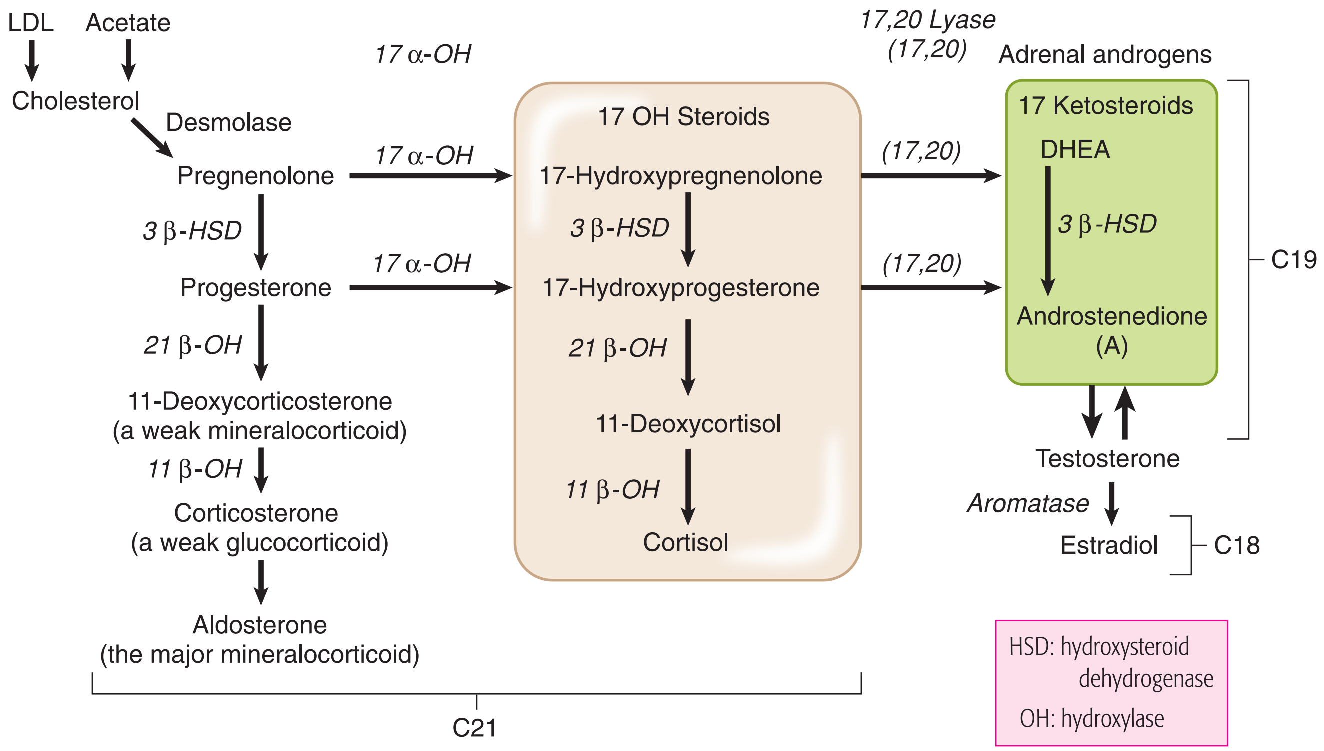


Figure VII-4-2. Pathways of Adrenal Steroid Synthesis

C21 steroids (21 carbon atoms)

C21 steroids with an OH at position 17 are called 17-hydroxysteroids. The only 17 OH steroid with hormonal activity is cortisol.

The lipid-soluble 17 OH steroids are metabolized to water-soluble compounds before they are filtered and excreted in the urine. The pathway for cortisol is shown below.

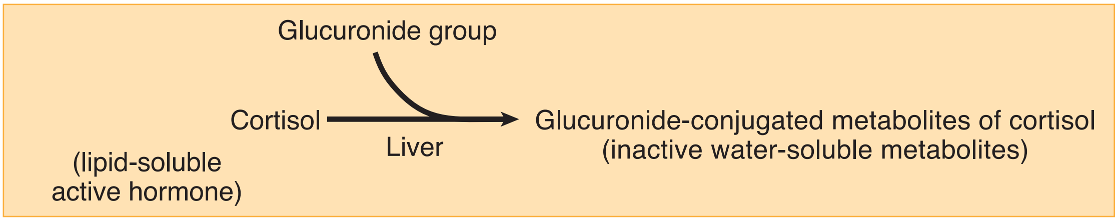


Figure VII-4-3. Metabolism of Cortisol

Urinary 17 OH steroids have in the past been measured as an index of cortisol secretion. This has been replaced by the measurement of the 24-hour urine-free cortisol.

C19 steroids (19 carbon atoms)

Adrenal Androgens

- Have a keto group at position 17 and are therefore called 17-ketosteroids.
- Are conjugated with sulfate in the adrenal cortex, making them water soluble. As water-soluble metabolites, they circulate in the bloodstream, are filtered by the kidney, and are excreted in the urine. The sulfated form is not produced in the gonads and is thus considered an index of androgen production by the adrenals.
- The major secreted form is dehydroepiandrosterone (DHEA).
- DHEA, DHEA sulfate, and androstenedione have very low androgenic activity. They function primarily as precursors for the peripheral conversion to the more potent testosterone and dihydrotestosterone (men and women).
- In adult males, excessive production of adrenal androgens has no clinical consequences. In prepubertal males it causes premature penile enlargement and early development of secondary sexual characteristics. In women excessive adrenal androgens cause hirsutism and virilization.

Testosterone

- Produced mainly by the Leydig cells of testes
- The active hormone is lipid-soluble and not a 17-ketosteroid.
- When metabolized, it is converted to a 17-ketosteroid and conjugated to become water soluble. In this form, it is filtered and excreted by the kidney.

Urinary Excretion

- Urinary 17-ketosteroids are an index of all androgens, adrenal and testicular.
- In females and prepubertal males, urinary 17-ketosteroids are an index of adrenal androgen secretion.
- In adult males (postpuberty), urinary 17-ketosteroids are 2/3 adrenal and 1/3 testicular, and thus mainly an index of adrenal secretion.

C18 steroids—estrogens (e.g., estradiol)

- Aromatase converts androgen into estrogen.

Regional Synthesis

Conversion of cholesterol to pregnenolone

The starting point in the synthesis of all steroid hormones is the transport of cholesterol into the mitochondria by steroidogenic acute regulatory protein (StAR). This is the rate-limiting step.

The enzyme catalyzing the conversion of cholesterol to pregnenolone is sidechain cleavage enzyme (SCC, also called desmolase).

Synthesis in the zona glomerulosa

The figure below represents the pathways present in the zona glomerulosa. Angiotensin II is the main stimulus to the zona glomerulosa, which produces aldosterone, the major mineralocorticoid.

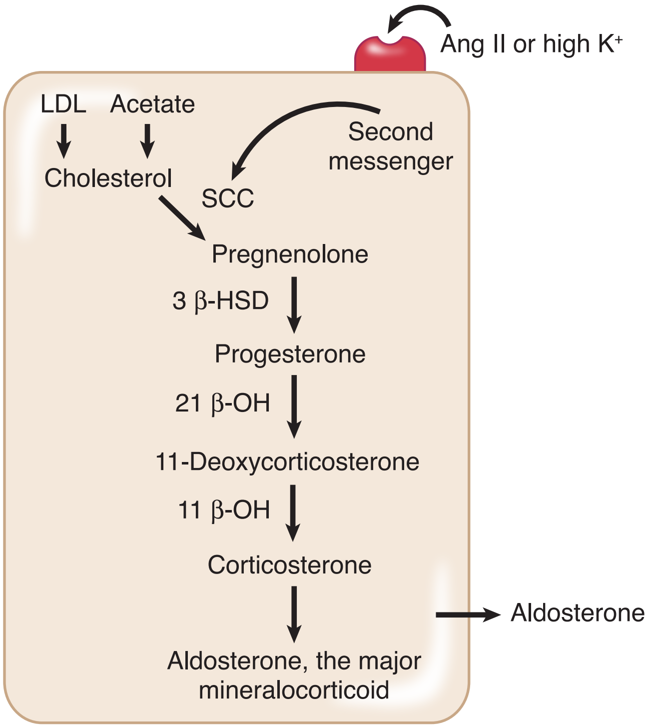


Figure VII-4-4. Pathway to Aldosterone Synthesis

Synthesis in the zona fasciculata and the zona reticularis

Normal hormonal output of the zona fasciculata and zona glomerulosa consists of the following:

- 11-Deoxycorticosterone: Under normal conditions, this weak mineralocorticoid is not important. Almost all mineralocorticoid activity is due to aldosterone.
- Corticosterone: Also not important under normal conditions. Almost all glucocorticoid activity is due to cortisol.
- Cortisol: Main glucocorticoid secreted by the adrenal cortex, responsible for most of the hypothalamic and anterior pituitary negative feedback control of ACTH secretion.

Normal hormonal output of the zona reticularis consists of the following:

- Adrenal androgens: These weak water-soluble androgens represent a significant secretion; however, they produce masculinizing characteristics only in women and prepubertal males when secretion is excessive.

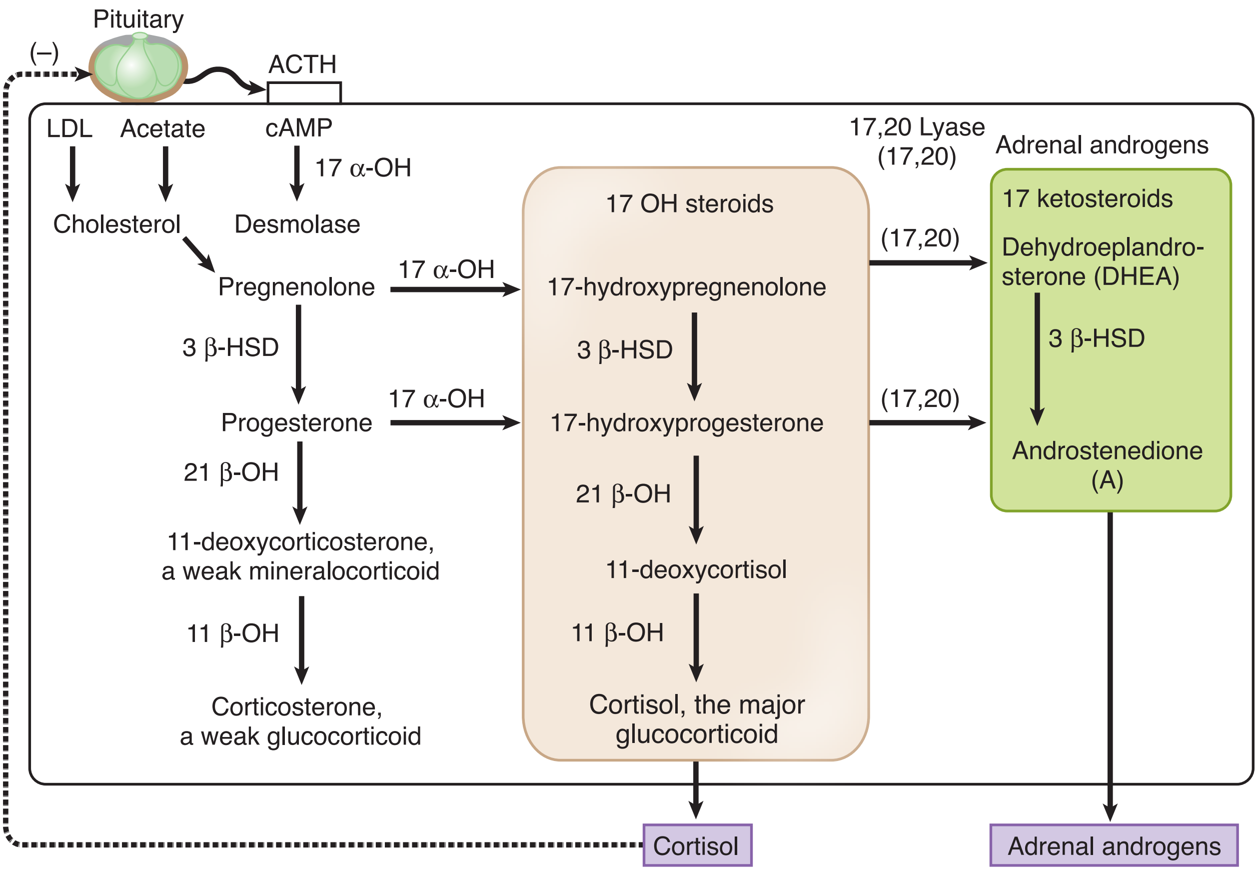


Figure VII-4-5. Control of Steroid Hormone Synthesis in the Zonas Fasciculata and Reticularis

PHYSIOLOGIC ACTIONS OF GLUCOCORTICOIDS

Stress

Stress includes states such as trauma, exposure to cold, illness, starvation, and exercise. The capacity to withstand stress is dependent on adequate secretion of the glucocorticoids.

Stress hormones usually act to mobilize energy stores. The stress hormones are:

- **Growth hormone:** mobilizes fatty acids by increasing lipolysis in adipose tissue
- **Glucagon:** mobilizes glucose by increasing liver glycogenolysis
- **Cortisol:** mobilizes fat, protein, carbohydrate (see below)
- **Epinephrine,** in some forms of stress such as exercise: mobilizes glucose via glycogenolysis and fat via lipolysis

All stress hormones raise plasma glucose. Severe hypoglycemia is a crisis and causes a rapid increase in all stress hormones. By definition, because these hormones raise plasma glucose, they are referred to as counterregulatory hormones (opposite to insulin).

A deficiency in a stress hormone may cause hypoglycemia.

Metabolic Actions of Cortisol

Cortisol promotes the mobilization of energy stores, specifically:

- **Protein:** Cortisol promotes degradation and increased delivery of hepatic gluconeogenic precursors.
- **Lipids:** Cortisol promotes lipolysis and increased delivery of free fatty acids and glycerol.
- **Carbohydrate:** Cortisol raises blood glucose, making more glucose available for nervous tissue. Two mechanisms are involved:
  - Cortisol counteracts insulin's action in most tissues (muscle, lymphoid, and fat).
  - Cortisol increases hepatic output of glucose by regulating the enzymes involved in gluconeogenesis, particularly phosphoenolpyruvate carboxykinase (PEPCK) (not from liver glycogenolysis).

Permissive Actions of Cortisol

Cortisol enhances the capacity of glucagon and catecholamines, hence the adjective *permissive* aptly describes many of the actions of cortisol.

Glucagon

Promotes glycogenolysis in the liver (some lipolysis from adipocytes as well). Without cortisol, fasting hypoglycemia rapidly develops. Cortisol permits glucagon to raise blood glucose.

Catecholamines

Promotes both alpha and beta receptor expression. Beta receptor function involves glucose regulation, lipolysis (see next chapter), and bronchodilation. Alpha receptor function is pivotal for blood pressure regulation. Without cortisol, blood pressure decreases.

CONTROL OF ADRENOCORTICOTROPIN (ACTH) AND CORTISOL SECRETION

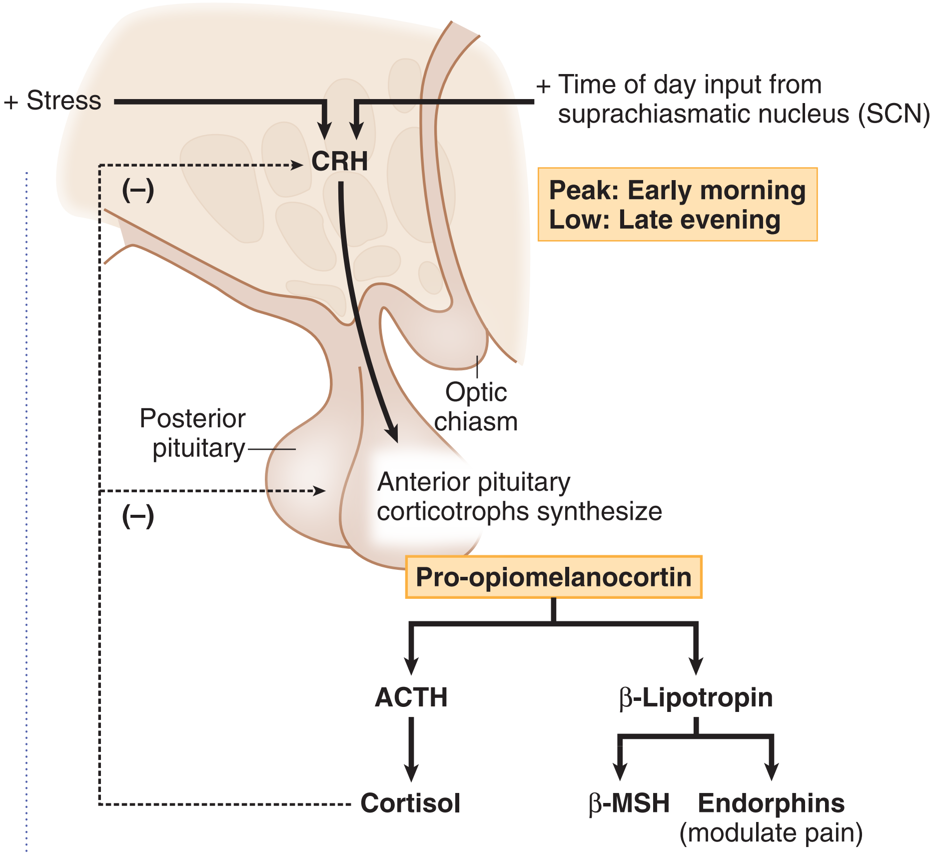


Figure VII-4-6. Control of ACTH and Cortisol

Role of the Specific Modulators

Secretion of **corticotropin-releasing hormone** (CRH) increases in response to stress and in the early morning:

- Peak cortisol secretion occurs early in the morning between the 6th and 8th hours of sleep. Secretion then declines slowly during the day and reaches a low point late in the evening.
- Increased AM CRH = increased AM cortisol
- Increased AM cortisol = increased AM blood sugar and lipid levels
- Increased AM sugar and lipid levels help get you out of bed

ACTH stimulates the secretion of cortisol (and adrenal androgens) of adrenal cortex. Cortisol suppresses the release of ACTH by acting on the hypothalamus and anterior pituitary.

Excessive secretion of ACTH (e.g., primary adrenal insufficiency) causes darkening of the skin. This is due to the melanocyte-stimulating hormone (α-MSH) sequence within the ACTH molecule, and the β-MSH activity of β-lipotropin.

β-lipotropin has a role not well-understood. It is a precursor to β-MSH and endorphins. Endorphins modulate the perception of pain.

PHYSIOLOGIC ACTIONS OF ALDOSTERONE

The primary target tissue for aldosterone is the kidney, where it increases Na^+ reabsorption by the principal cells of the kidney's collecting ducts. Because water is reabsorbed along with the Na^+, aldosterone can be considered to control the amount of Na^+ rather than the concentration of Na^+ in the ECF.

Aldosterone also promotes the secretion of H^+ by the intercalated cells of the collecting duct, and K^+ secretion by the principal cells. The Na^+-conserving action of aldosterone is also seen in salivary ducts, sweat glands, and the distal colon.

The figure below shows the overall effects of aldosterone. This is a generalized representation of the effect of aldosterone on the renal distal tubule/collecting duct region.

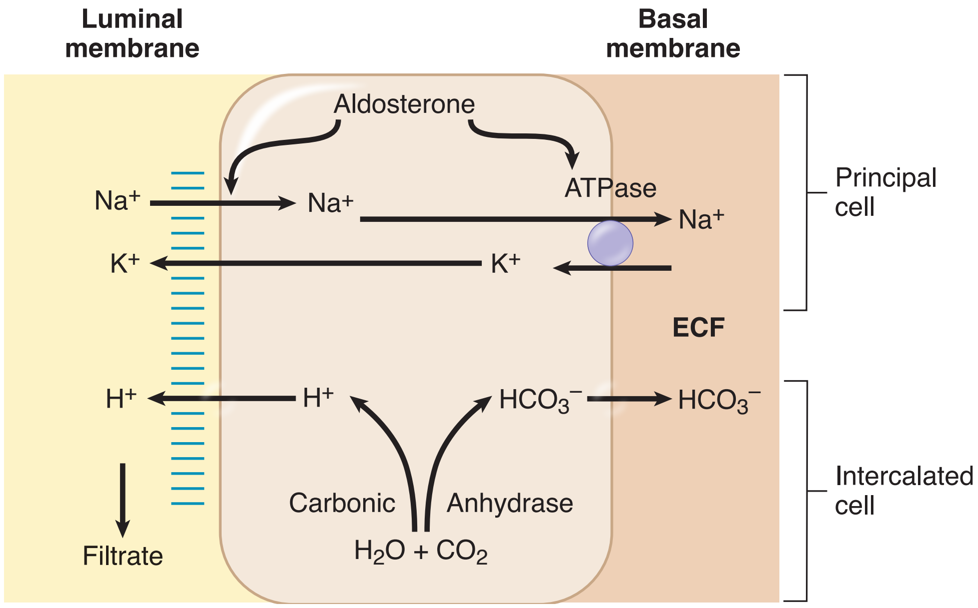


Figure VII-4-7. Late Distal Tubule and Collecting Duct

Specific Actions of Aldosterone

Aldosterone promotes the activity of Na/K-ATPase–dependent pump that moves Na^+ into the renal ECF in exchange for K^+. In addition, aldosterone enhances epithelial Na^+ channels (ENaC) in the luminal membrane of principal cells. The net effect is to increase Na^+ reabsorption, which in turn increases water reabsorption. Aldosterone regulates Na^+ to regulate extracellular volume.

The reabsorption of Na^+ creates a negative luminal potential promoting K^+ excretion.

Aldosterone stimulates H^+ secretion by intercalated cells. Thus, excess aldosterone causes alkalosis, while insufficient aldosterone causes acidosis (type IV RTA).

Table VII-4-1. Actions of Aldosterone

	Renal	
Na^+	reabsorption	↑ total body Na^+
K^+	secretion	↓ plasma [K^+]
H^+	secretion	promotes metabolic alkalosis
HCO_3^-	production	promotes metabolic alkalosis
H_2O	reabsorption	volume expansion

CONTROL OF ALDOSTERONE SECRETION

Controlling Factors

Acutely, ACTH increases aldosterone secretion. However, the primary regulators of aldosterone secretion are circulating levels of Ang II and K^+.

Sensory Input—the Juxtaglomerular Apparatus

The main sensory cells are the granular cells (also called juxtamedullary cells) of the afferent arteriole. They are modified smooth-muscle cells that surround and directly monitor the pressure in the afferent arteriole. This signal in many cases is in response to a reduction in circulating fluid volume.

These cells are also innervated and stimulated by sympathetic neurons via norepinephrine and beta receptors. Thus the release of renin induced by hypovolemia is enhanced by increased sympathetic neural activity.

Additional sensory input is from the macula densa cells of the distal tubule. They perceive sodium delivery to the distal nephron and communicate with the juxtaglomerular cells.

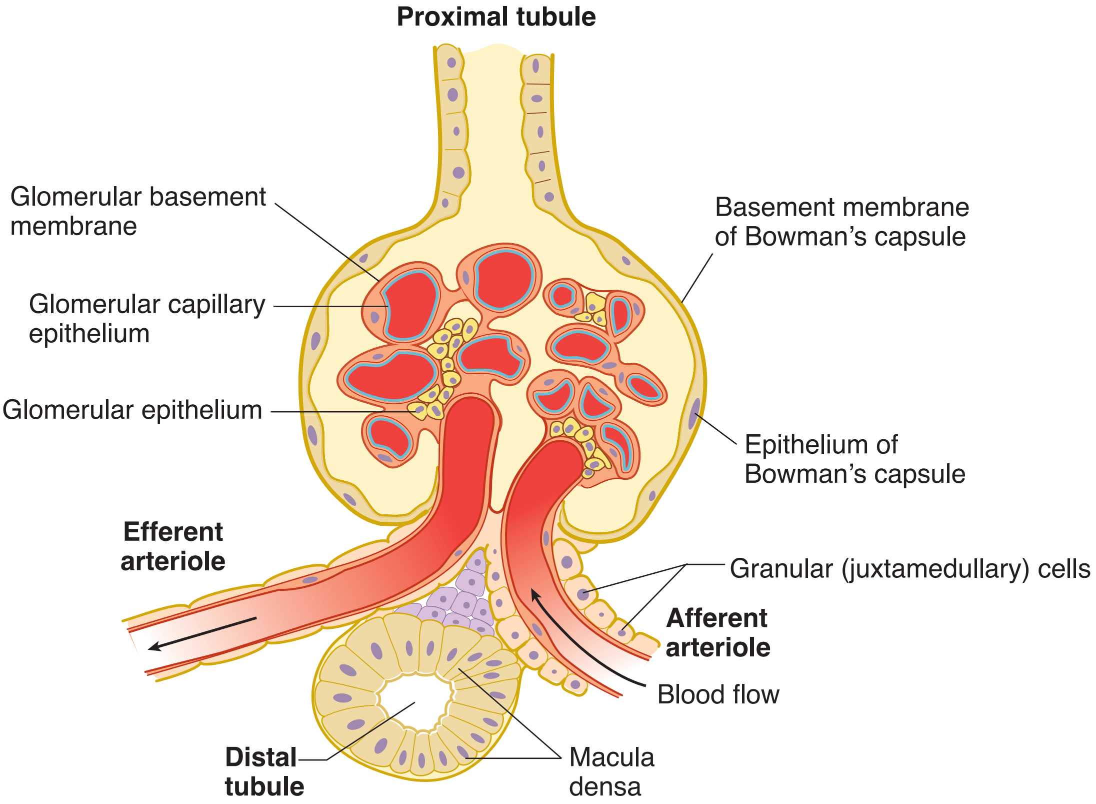


Figure VII-4-8. Renal Corpuscle and Juxtaglomerular Apparatus

Long-Term Regulation of Blood Pressure and Cardiac Output by the Renin-Angiotensin-Aldosterone System

Long-term regulation of blood pressure and cardiac output is accomplished by the renin-angiotensin-aldosterone system.

Blood pressure is monitored by the juxtaglomerular apparatus. When renal perfusion pressure decreases, secretion of renin increases; conversely, when pressure increases, renin secretion is suppressed. Renin is an enzyme that converts a circulating protein produced in the liver, **angiotensinogen** into **angiotensin I**. Angiotensin converting enzyme (ACE), found mainly in endothelial cells of pulmonary vessels, converts angiotensin I into **angiotensin II**. Angiotensin II has potent effects to stimulate secretion of aldosterone and to cause arteriolar vasoconstriction. It also directly stimulates reabsorption of sodium in the proximal tubule.

$$\mathbf{MAP = CO \times TPR}$$

This system regulates both resistance, via vasoconstriction, and cardiac output, via preload. Since aldosterone also causes increased renal excretion of potassium, it affects plasma potassium concentration. Plasma potassium strongly stimulates secretion of aldosterone, so this constitutes a negative-feedback control system for plasma potassium concentration.

Volume-depleted states tend to produce metabolic alkalosis, in part because aldosterone increases to compensate for the volume loss; the aldosterone increase stimulates excretion of acid and addition of bicarbonate to the plasma.

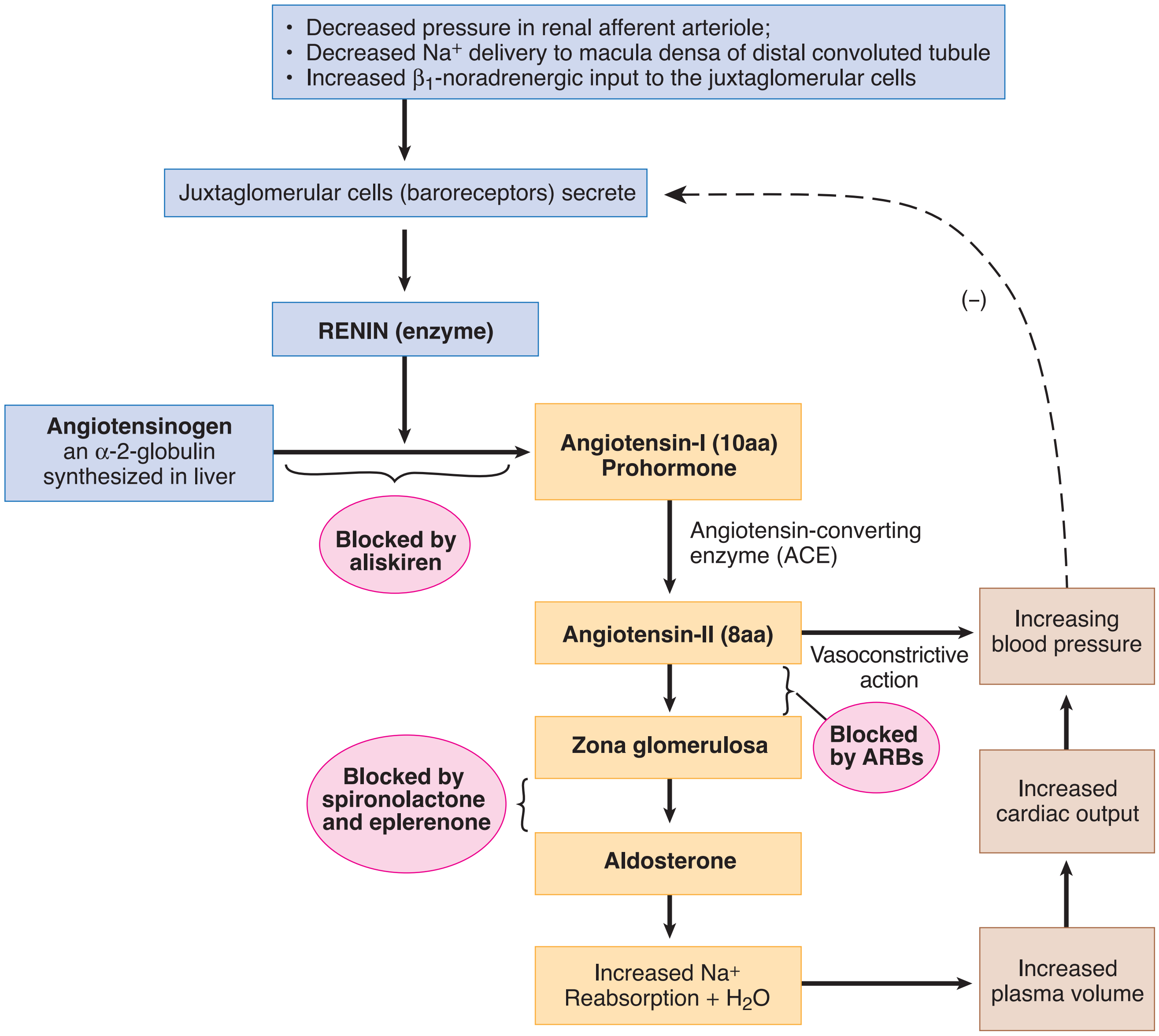


Figure VII-4-9. Feedback Control of Blood Pressure by Renin-Angiotensin-Aldosterone System

Any of the 3 stimuli listed at the top of the figure produces an increase in the secretion of renin and circulating angiotensin II. Angiotensin II raises blood pressure by 2 independent actions:

- The direct vasoconstrictive effects of angiotensin II increase TPR.
- It stimulates the adrenal cortex to secrete aldosterone, resulting in increased reabsorption of Na^+.

As Na^+ reabsorption is increased, so is water. This increases the volume of the ECF, thus raising cardiac output and blood pressure.

An increase in blood pressure suppresses the renin-angiotensin-aldosterone system (RAAS). This decrease in angiotensin II reduces TPR. Reduced activity of aldosterone causes a urinary loss of sodium and water, lowering cardiac output.

In addition to its effects to serve as a direct vasoconstrictor and increase aldosterone secretion, angiotensin II also:

- Increases ADH release from posterior pituitary
- Increases thirst
- Increases sodium reabsorption in proximal tubule

Potassium Effect

In addition to angiotensin II, elevated plasma K^+ also increases aldosterone. This is negative feedback regulation of K^+ (*see* Renal Section).

GLUCOCORTICOID DISORDERS

Definitions

Cushing syndrome: hypercortisolism regardless of origin, including chronic glucocorticoid therapy

Cushing disease: hypercortisolism due to an adenoma of the anterior pituitary (microadenoma)

The first step in the evaluation of possible hypercortisolism is to establish that the cortisol level is truly elevated. Once this is done, the ACTH level and high-dose dexamethasone suppression tests are used to determine the source or etiology of the hypercortisolism.

Establishing the Presence of Hypercortisolism

First do a 24-hour urine-free-cortisol or 1 mg overnight dexamethasone suppression test. A single random cortisol level should not be used to diagnose hypercortisolism.

24-hour urine-free-cortisol is harder to do but it has fewer falsely positive tests.

1-mg overnight dexamethasone suppression test

- For the presence of Cushing syndrome regardless of the cause
- Normal; cortisol decreases
- Hypercortisolism; cortisol not suppressed
- False-positives from depression or alcoholism

High-dose dexamethasone

- To differentiate pituitary adenoma from ectopic ACTH secretion and adrenal tumors
- Pituitary source; cortisol decreases
- Ectopic ACTH, adrenal tumor; cortisol not suppressed

ACTH level

- Used after 24 hour urine cortisol establishes presence of hypercortisolism
- ACTH levels establish etiology of hypercortisolism
- Low ACTH = Adrenal source of cortisol overproduction
- Normal or high ACTH = pituitary or ectopic source
- High dose dexamethasone distinguishes pituitary vs ectopic source

Note

Hypercortisolism and an ACTH that is in the normal range or high is a secondary condition. Via negative feedback, a high cortisol should produce a low ACTH. If ACTH is in the "normal" range, then it is "inappropriately" normal (meaning it is too high) and is thus the cause of the hypercortisolism.

Stimulation Tests

ACTH stimulation test diagnoses adrenal insufficiency.

- To diagnose atrophied adrenal nonresponsive
- **Normal;** cortisol increases after ACTH
- Adrenal insufficiency: no change in cortisol level

Clinical Correlate

Metyrapone testing is no longer performed.

- Metyrapone is no longer manufactured
- Simulates 11 beta-hydroxylase deficiency
  - Normal = ACTH **goes up**
  - Pituitary insufficiency = ACTH **fails to rise**

Hypercortisolism

Primary hypercortisolism (adrenal source)

- ACTH independent
- Cortisol elevated, ACTH depressed
- Most are benign adrenocorticol adenomas
- Adrenal adenoma usually unilateral and secretes only cortisol; decreased adrenal androgen and deoxycorticosterone (hirsutism absent)
- Presence of androgen or mineralocorticoid excess suggests a carcinoma.

Secondary hypercortisolism (pituitary vs. ectopic source)

- ACTH dependent
- Hypersecretion of ACTH results in bilateral hyperplasia of the adrenal zona fasciculata and reticularis
- Elevated ACTH, cortisol, adrenal androgen, deoxycorticosterone
- Two main subcategories:
  - **Pituitary adenoma,** usually a microadenoma (< 1 cm dia.)
    - This is Cushing disease
    - Increased ACTH not sufficient to cause hyperpigmentation
    - Dexamethasone suppressible

- **Ectopic ACTH syndrome:**
  - Most frequently in patients with small cell carcinoma of the lung
  - Greater secretion of ACTH than in Cushing disease and hyperpigmentation often present
  - Ectopic site nonsupressible with dexamethasone

Differential diagnosis

- Hypercortisolism established by lack of cortisol suppression to 1 mg overnight dexamethasone and/or elevated 24-hour urine free cortisol
- Decreased plasma ACTH: Adrenal is source (primary hypercortisolism)
- High-dose dexamethasone
  - ACTH suppressed = Cushing disease (pituitary source)
  - ACTH not suppressed = ectopic ACTH syndrome

Characteristics of Cushing syndrome

- Obesity because of hyperphagia, classically central affecting mainly the face, neck, trunk, and abdomen: "moon facies" and "buffalo hump"
- Protein depletion as a result of excessive protein catabolism
- Inhibition of inflammatory response and poor wound healing
- Hyperglycemia leads to hyperinsulinemia and insulin resistance.
- Hyperlipidemia
- Bone breakdown and osteoporosis
- Thinning of the skin with wide purple striae located around abdomen and hips
- Increased adrenal androgens, when present in women, can result in acne, mild hirsutism, and amenorrhea. In men, decreased libido and impotence
- Mineralocorticoid effects of the high level of glucocorticoid and deoxycorticosteroid lead to salt and water retention (hypertension), potassium depletion, and a hypokalemic alkalosis.
- Increased thirst and polyuria
- Anxiety, depression, and other emotional disorders may be present.

Hypocortisolism

Primary hypocortisolism (in primary adrenal insufficiency, Addison's disease)

Cortisol deficiency leads to weakness, fatigue, anorexia, weight loss, hypotension, hyponatremia, hypoglycemia. Increases in ACTH result in hyperpigmentation of skin and mucous membranes.

Aldosterone deficiency leads to sodium wasting and hyponatremia, potassium retention and hyperkalemia, dehydration, hypotension, and acidosis

- Autoimmune origin with slow onset in about 80% of cases
- Loss of 90% of both adrenals required before obvious clinical manifestations
- With gradual adrenal destruction, basal secretion is normal but secretion does not respond to stress, which may initiate an adrenal crisis.
- Bilateral hemorrhage as the origin results in an adrenal crisis. Hyperpigmentation, hyponatremia, and hyperkalemia usually absent
- Orthostatic intolerance due to diminished alpha-receptor function and low blood volume.
- Abnormalities in GI function
- Loss of axillary and pubic hair in women due to loss of androgens, amenorrhea
- Insufficient glucocorticoids leads to hypoglycemia and an inability of the kidney to excrete a water load
- Severe hypoglycemia in children but rare in adults

Secondary hypocortisolism (secondary adrenal insufficiency)

- Most commonly due to sudden withdrawal of exogenous glucocorticoid therapy
- Trauma, infection, and infarction most common natural origin of ACTH deficiency
- In the early stages baseline hormone values are normal but ACTH reserve compromised and stress response subnormal (glucocorticoids administered presurgery)
- May be associated with the loss of other anterior pituitary hormones (panhypopituitarism) or adenomas secreting prolactin or growth hormone
- Atrophy of the zona fasciculata and zona reticularis
- Zona glomerulosa and aldosterone normal; no manifestations of mineralocorticoid deficiency
- Consequences as stated for cortisol deficiency
- Severe hypoglycemia and severe hypotension unusual (RAAS is still intact)
- Hyponatremia due to water retention

Differential diagnosis

- Rapid ACTH stimulation test: initial procedure in the assessment of adrenal insufficiency, both primary and secondary.
  - Normal: ↑ cortisol
  - Hypocortisolism: diminished ↑ cortisol
- Normal responsiveness of ACTH test does not exclude decreased pituitary reserve and decreased response to stress (insulin infusion)

- In same sample, a normal aldosterone would be evidence of a secondary problem
- Definitive test for primary vs. secondary is ACTH: ↑ primary hypocortisolism (Addison's); inappropriately low in secondary hypocortisolism

Summary

Table VII-4-2. Primary and Secondary Disorders of Cortisol Secretion

Disorder	Plasma Cortisol	Plasma ACTH	Hyperpigmentation
Primary hypercortisolism	↑	↓	no
Secondary hypercortisolism			
Cushing disease	↑	normal or ↑	no
Ectopic ACTH	↑	↑	yes (maybe)
Primary hypocortisolism	↓	↑	yes
Secondary hypocortisolism	↓	↓	no

MINERALOCORTICOID DISORDERS

Hyperaldosteronism with Hypertension

Primary hyperaldosteronism (Conn's syndrome)

- Most common cause is a small unilateral adenoma, on either side
- Remainder mostly bilateral adrenal hyperplasia (idiopathic hyperaldosteronism)
- Rarely due to adrenal carcinoma
- Increased whole body sodium, fluid, and circulating blood volume
- Hypernatremia is infrequent
- Increased peripheral vasoconstriction and TPR
- Blood pressure from borderline to severe hypertension
- Edema rare (sodium escape*)
- Modest left ventricular hypertrophy

*A major increase in sodium and water retention is prevented by "sodium escape" in primary hyperaldosteronism. The mechanism for this escape is still unclear.

- Potassium depletion and hypokalemia create symptoms of weakness and fatigue.
- Detection of hypertension with hypokalemia is often the initial clue for Conn's syndrome
- Increased hydrogen ion excretion and new bicarbonate create metabolic alkalosis.

- A positive Chvostek or Trousseau's sign is suggestive of alkalosis leading to low calcium levels.
- Cortisol is normal.
- Suppression of renin a major feature

Secondary hyperaldosteronism refers to a state in which there is an appropriate increase in aldosterone in response to activation of the renin-angiotensin system.

Secondary hyperaldosteronism with hypertension

- In most cases a primary over-secretion of renin secondary to a decrease in renal blood flow and/or pressure
- Renal arterial stenosis, narrowing via atherosclerosis, fibromuscular hyperplasia.
- Renin-secreting tumor rare
- Modest to highly elevated renin
- Modest to highly elevated aldosterone
- Hypokalemia and metabolic alkalosis

Differential diagnosis

- Hypokalemia in a hypertensive patient not taking diuretics
- Hyposecretion of renin with elevated aldosterone that fails to respond to a volume contraction: Conn's syndrome
- Hypersecretion of renin with elevated aldosterone: renal vascular

Hyperaldosteronism with Hypotension

Secondary hyperaldosteronism with hypotension

Sequestration of blood on the venous side of the systemic circulation is a common cause of secondary hyperaldosteronism. This results in decreased cardiac output and thus decreased blood flow and pressure in the renal artery. The following conditions produce secondary hyperaldosteronism through this mechanism:

- Congestive heart failure
- Constriction of the vena cava
- Hepatic cirrhosis

Table VII-4-3. Secondary Hyperaldosteronism

The cause in all cases is a **decrease** in blood pressure.	
1. Plasma renin and angiotensin II activity: The increased angiotensin II activity will drive the secondary hyperaldosteronism.	↑
2. Total body sodium:	↑
3. ECF volume:	↑
4. Plasma volume:	↑
5. Edema*:	yes

*Na^+ escape prevents peripheral edema in primary but not secondary hyperaldosteronism. Also note that the increased ECF volume remains mainly on the venous side of the circulation, accentuating the venous congestion and preventing a return of circulating blood volume to normal.

ENZYME DEFICIENCIES

Single enzyme defects can occur as congenital "inborn errors of metabolism." Congenital defects in any of the enzymes lead to **deficient cortisol secretion** and the syndrome called *congenital adrenal hyperplasia*. Hyperplasia is caused by the excessive secretion of ACTH that results from the loss of the negative feedback action of cortisol.

In all the following examples, assume the deficiency is significant to the extent that it affects normal hormonal production but not a complete blockade.

A useful summary of enzyme deficiency conditions is that a horizontal cut of the pathway causes decreased production of all substances below the cut and increased secretion of substances above the cut. A vertical cut causes decrease of substances to the right of the cut and increase of substances to the left of the cut.

21 β-Hydroxylase Deficiency

21 β-hydroxylase deficiency is the most common of the congenital enzyme deficiencies.

Tissues affected: zona glomerulosa, zona fasciculata, zona reticularis

Effect in the zona glomerulosa

The **blockade point** in the zona glomerulosa can be seen below.

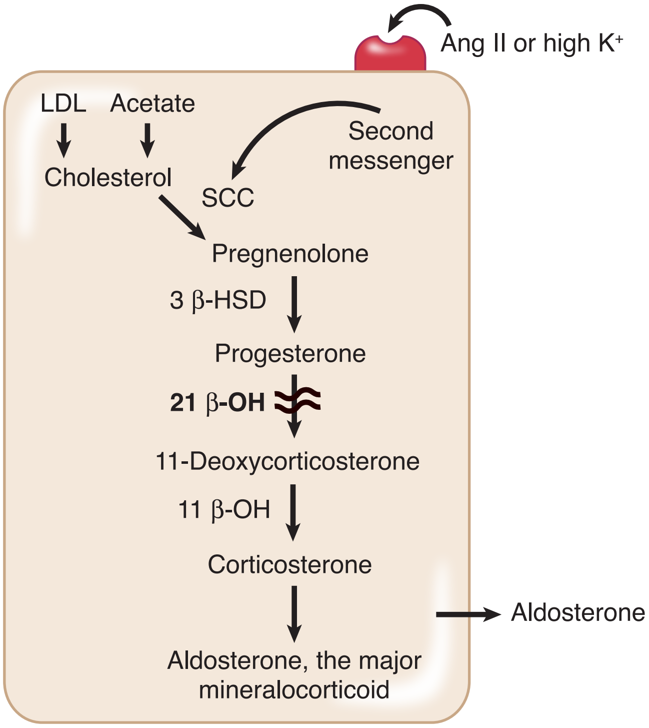


Figure VII-4-10. 21-β Enzyme Deficiency in the Zona Glomerulosa

Consequence: Result is a decreased production of aldosterone, the main mineralocorticoid.

Cholesterol can be made "de novo" from acetate if there is nutritional deficiency.

Effect in the zona fasciculata and zona reticularis

The 2 **blockade points** (wavy lines) in the zona fasciculata and zona reticularis can be seen below.

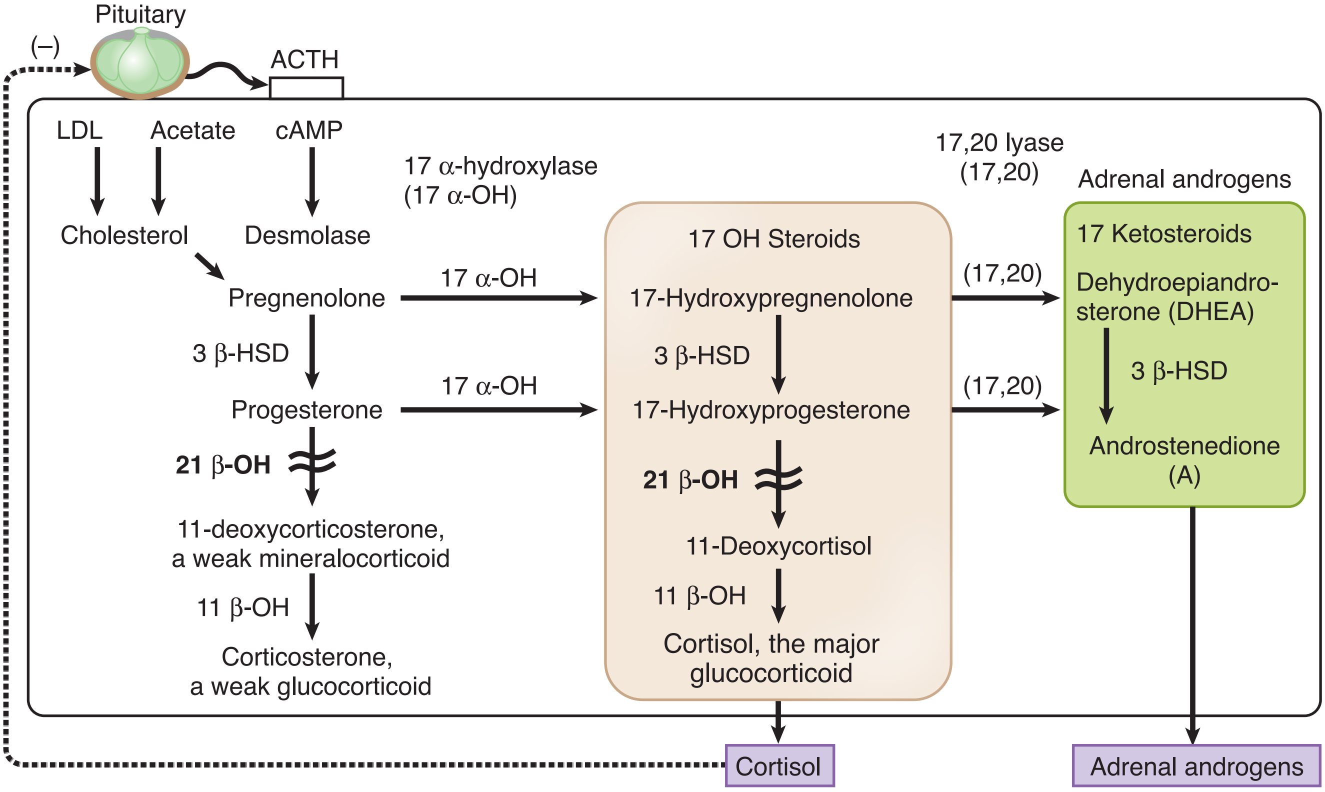


Figure VII-4-11. 21-β Enzyme Deficiency in the Zona Fasciculata and Zona Reticularis

Summary of overall pathway changes:

- Zona glomerulosa: decreased aldosterone
- Zona fasciculata, reticularis: decreased production of 11-deoxycorticosterone, a weak mineralocorticoid.
- Therefore, a mineralocorticoid deficiency, loss of Na^+, volume and a hypotensive state (salt-wasting state).
- Increased renin secretion and increased circulating angiotensin II.
- Decreased production of corticosterone, a weak glucocorticoid, and cortisol.
- Therefore, glucocorticoid deficiency and increased ACTH, which drive increases in adrenal androgen secretion

11 β-Hydroxylase Deficiency

Tissues affected: zona fasciculata, zona reticularis, zona glomerulosa

Effect in the zona fasciculata and zona reticularis

The **blockade** in the zona fasciculata and zona reticularis can be seen below.

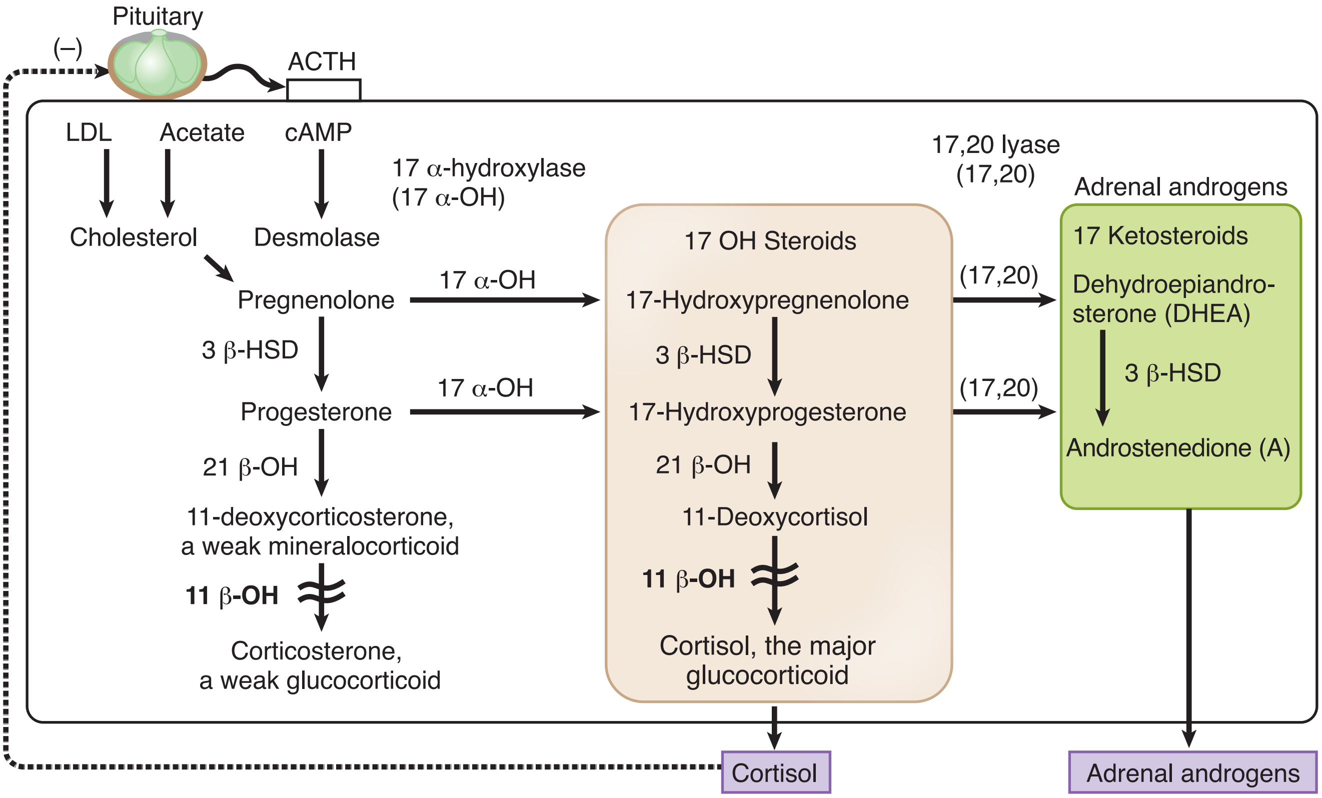


Figure VII-4-12. 11-β-Hydroxylase Deficiency in the Zona Fasciculata and Zona Reticularis

Deoxycorticosterone increases blood pressure. Only cortisol is feedback inhibition of the pituitary for ACTH.

Effect in the zona glomerulosa

The effect on the zona glomerulosa can be seen below.

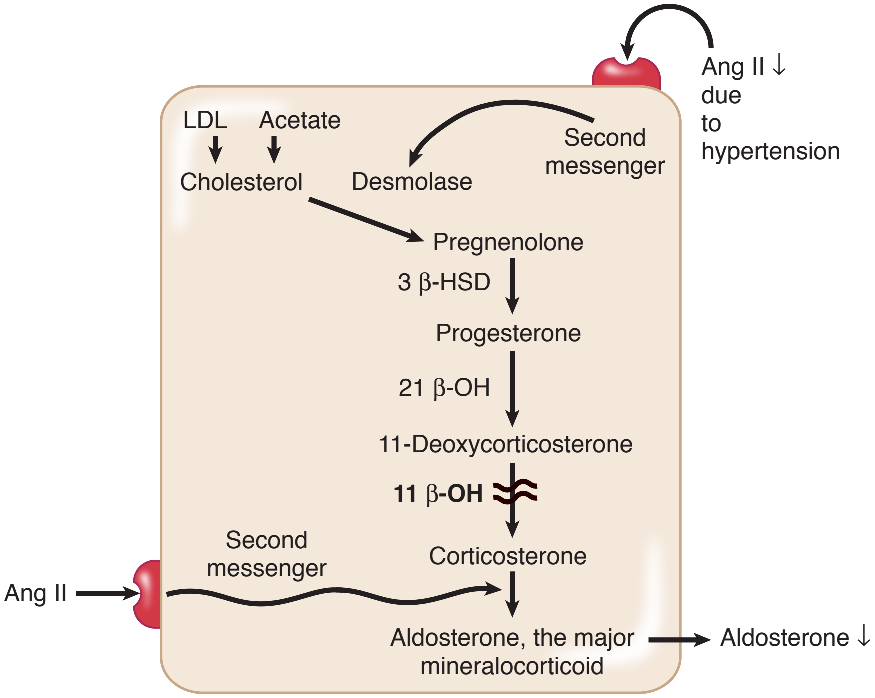


Figure VII-4-13. 11-β-Hydroxylase Deficiency in the Zona Glomerulosa

Summary of overall pathway changes:

- Zona fasciculata, reticularis: decreased corticosterone and cortisol, increased ACTH and overproduction of steroids above the blockade, including:
  - Androgens and the consequences in women and prepubertal males
  - 11-deoxycorticosterone, a mineralocorticoid that leads to hypertension and a decrease in circulating angiotensin II
- Zona glomerulosa: decreased aldosterone because of loss of necessary enzyme and low angiotensin II

17 α-Hydroxylase Deficiency

Tissues affected: zona fasciculata, zona reticularis, testis, ovary

Blockade in the adrenal zona fasciculata and the zona reticularis

The blockade points in the zona fasciculata and zona reticularis can be seen below.

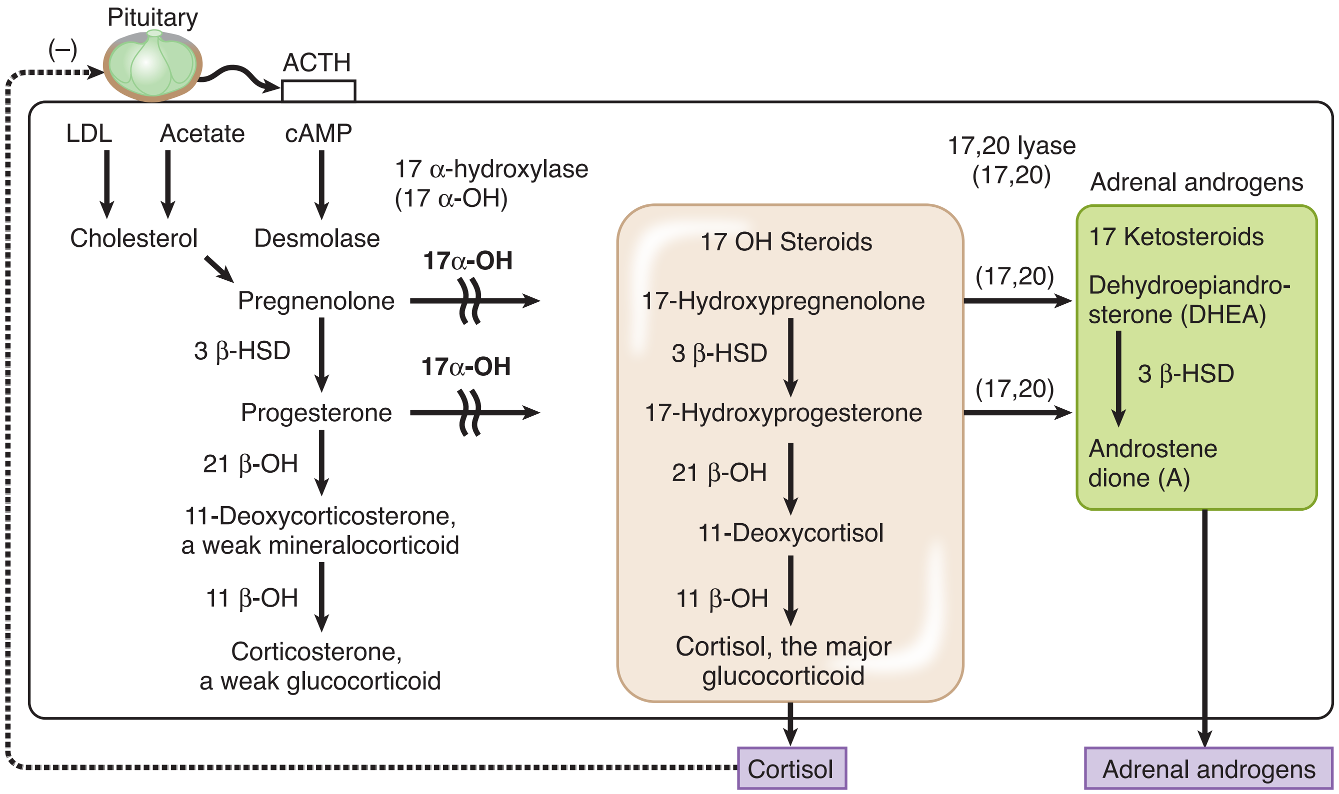


Figure VII-4-14. 17 α-Hydroxylase Deficiency

Summary of overall pathway changes:

- Zona fasciculata, reticularis: decreased adrenal androgens, decreased cortisol, and increased ACTH. Increased 11-deoxycorticosterone leading to hypertension. The reduced circulating angiotensin II reduces stimulation of zona glomerulosa and aldosterone secretion.

Effect in the testes

The blockade points in the testes can be seen below.

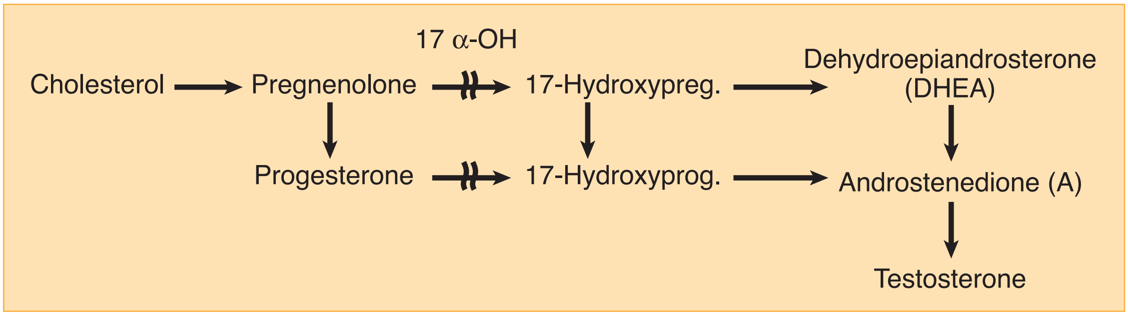


Figure VII-4-15. Testicular 17 α-OH Deficiency

Summary: decreased production of all androgens including testosterone

Effect in the ovaries

The blockade points in the ovaries can be seen below.

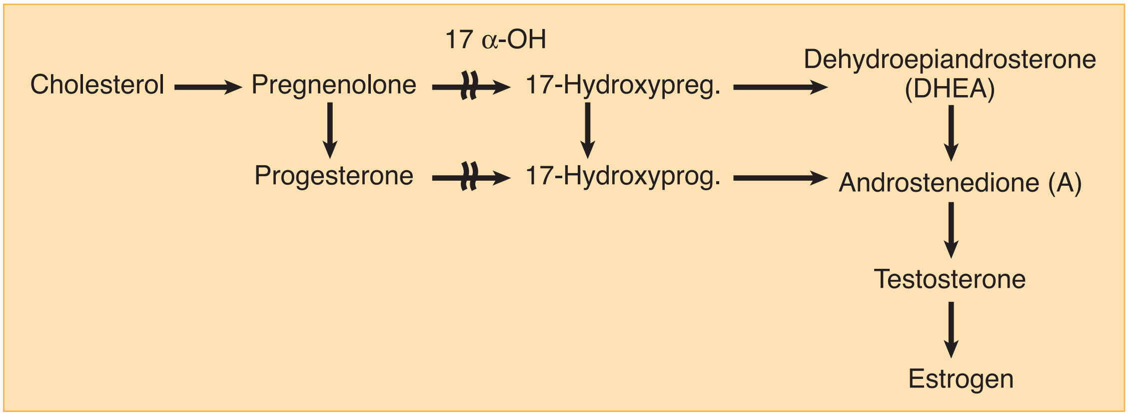


Figure VII-4-16. Ovarian 17 α-OH Deficiency

Summary: decreased production of estrogens and androgens

Summary

Table VII-4-4. Enzyme Deficiencies

Deficiency	Glucocorticoid	ACTH	Blood Pressure	Mineralocorticoid Aldo	Mineralocorticoid DOC	Androgen	Estrogen
21 β-OH	↓	↑	↓	↓	↓	↑ adrenal	_
11 β-OH	↓	↑	↑	↓	↑	↑ adrenal	_
17 α-OH	↓	↑	↑	↓	↑	↓ adrenal & testicular	↓

Note: In all 3 disorders, there will be a deficiency in cortisol and an increase in circulating ACTH. The ACTH is responsible for the adrenal hyperplasia.

Consequences of Congenital Adrenal Hyperplasia

21 β-Hydroxylase deficiency

21 β-hydroxylase deficiency accounts for about 90% of the cases.

- 75% of cases have mineralocorticoid deficiency
- Neonates may present with a salt-wasting crisis.
- Salt wasters tend to have hyponatremia, hyperkalemia, and raised plasma renin.
- 17-hydroxyprogesterone is elevated.
- Increased androgens lead to virilization of the female fetus and sexual ambiguity at birth
- Males are phenotypically normal at birth but develop precocious pseudopuberty, growth acceleration, premature epiphyseal plate closure, and diminished final height.
- Goal in treatment is to bring glucocorticoid and mineralocorticoid back to the normal range which would also suppress adrenal androgen secretion.
- Give hydrocortisone to act as feedback inhibition on pituitary.

11 β-Hydroxylase deficiency

- Clinical features of increased androgens similar to the preceding form, including virilization of female fetus.
- The principal difference with this form is the hypertension produced by 11-deoxycorticosterone, along with hypokalemia and suppressed renin secretion.
- Treatment for all forms of CAH is glucocorticoids such as hydrocortisone and dexamethasone.

17 α-Hydroxylase deficiency

- Extremely rare
- Usually diagnosed at the time of puberty when the patient presents with hypertension, hypokalemia, and hypogonadism
- Individuals have eunuchoid characteristics.

Recall Question

Which of the following would be seen on laboratory examination of a patient suffering from primary hypercortisolism?

A. Decreased plasma cortisol, decreased plasma ACTH, without hyperpigmentation

B. Decreased plasma cortisol, increased plasma ACTH, with hyperpigmentation

C. Increased plasma cortisol, increased plasma ACTH, with hyperpigmentation

D. Increased plasma cortisol, increased plasma ACTH, without hyperpigmentation

E. Increased plasma cortisol, decreased plasma ACTH, without hyperpigmentation

Answer: E

Adrenal Medulla 5

Learning Objectives

- ❏ Answer questions about hormones of the adrenal medulla
- ❏ Demonstrate understanding of major metabolic actions of epinephrine
- ❏ Interpret scenarios on pheochromocytomas

HORMONES OF THE ADRENAL MEDULLA

- Secretion of the adrenal medulla is 20% norepinephrine and 80% epinephrine.
- Phenylethanolamine-N-methyltransferase (PNMT) converts norepinephrine into epinephrine.
- Half-life of the catecholamines is only about 2 minutes. Metabolic endproducts include metanephrines and vanillylmandelic acid (VMA) both of which can be measured in plasma and urine
- Removal of the adrenal medulla reduces plasma epinephrine to very low levels but does not alter plasma norepinephrine. Most circulating norepinephrine arises from postganglionic sympathetic neurons.
- Because many of the actions of epinephrine are also mediated by norepinephrine, the adrenal medulla is not essential for life.
- The vasoconstrictive action of norepinephrine is essential for the maintenance of normal blood pressure, especially when an individual is standing. Plasma norepinephrine levels double when one goes from a lying to a standing position. People with inadequate production of norepinephrine suffer from orthostatic hypotension.
- Epinephrine is a stress hormone and rapidly increases in response to exercise, exposure to cold, emergencies, and hypoglycemia.

MAJOR METABOLIC ACTIONS OF EPINEPHRINE

- Liver: Epinephrine increases the activity of liver and muscle phosphorylase, promoting glycogenolysis. This increases glucose output by the liver.
- Skeletal muscle: Epinephrine promotes glycogenolysis but because muscle lacks glucose-6-phosphatase, glucose cannot be released by skeletal muscle; instead, it must be metabolized at least to lactate before being released into the circulation.
- Adipose tissue: Epinephrine increases lipolysis in adipose tissue by increasing the activity of hormone-sensitive lipase. Glycerol from TG breakdown is a minor substrate for gluconeogenesis.
- Epinephrine increases the metabolic rate. This will not occur without thyroid hormones or the adrenal cortex.

Metabolic Actions of Epinephrine on CHO and Fat

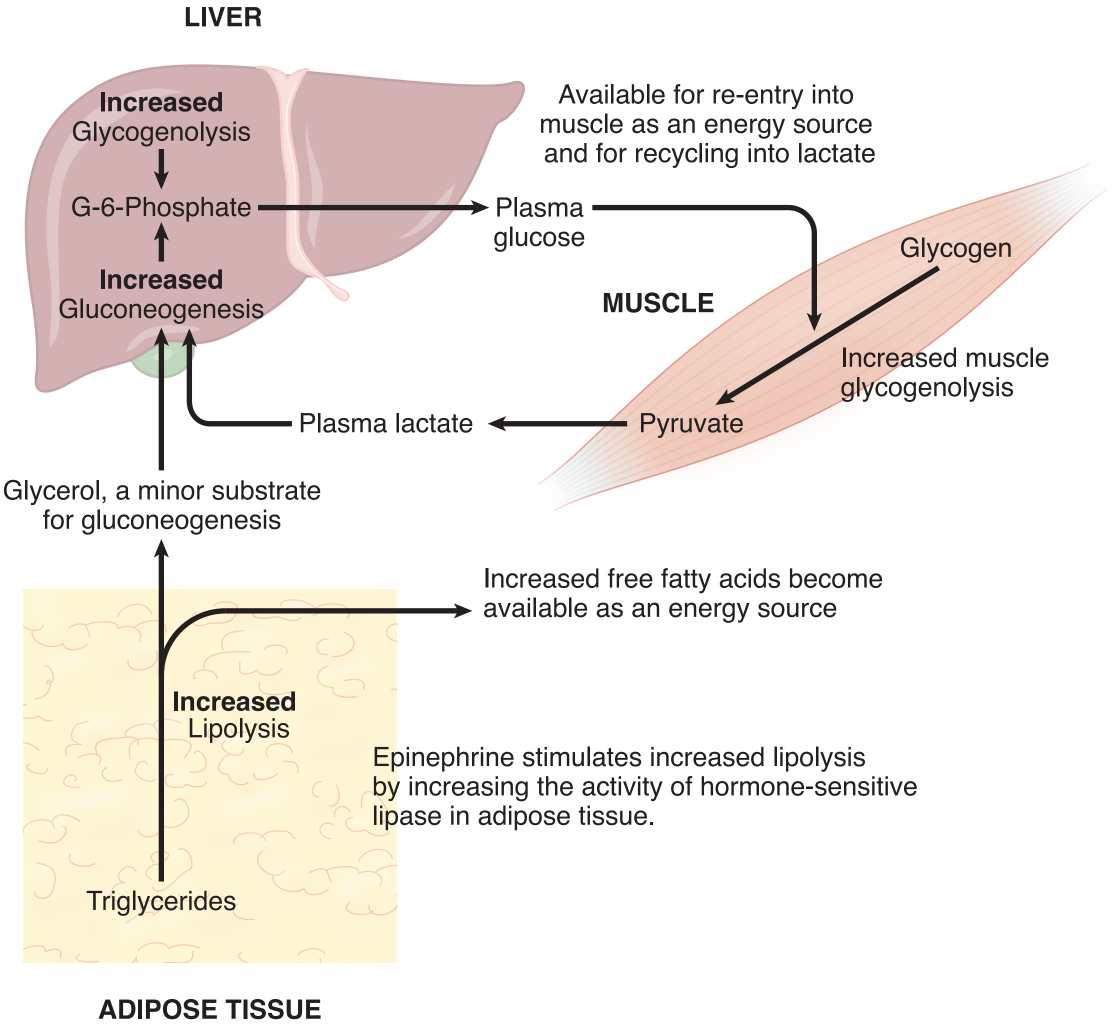


Figure VII-5-1. Actions of Catecholamines

PHEOCHROMOCYTOMAS

Pheochromocytomas are adrenal tumors that secrete epinephrine and norepinephrine in various ratios. They are usually unilateral benign tumors.

- Characteristic of MEN 2A and MEN 2B
- Paragangliomas are extra-adrenal pheochromocytomas of sympathetic ganglia located primarily within the abdomen and that secrete norepinephrine.
- Most consistent feature is hypertension. Symptoms include headache, diaphoresis, palpitations, and anxiety. Increased metabolic rate and hyperglycemia also occur.
- Pheochromocytomas are highly vascular and encapsulated.
- Episodic release of hormone, particularly when it is mainly norepinephrine, can abruptly cause a hypertensive crisis. Can be induced by physical stimuli that displaces abdominal contents.
- Most reliable initial test is plasma metanephrines or 24-hour urine catecholamines or metanephrines.
- Usually curable but can be fatal if undiagnosed
- Treat with alpha blocker followed by surgical removal.

Endocrine Pancreas 6

Learning Objectives

- ❏ Use knowledge of hormones of the islets of Langerhans
- ❏ Use knowledge of actions of insulin
- ❏ Use knowledge of control of insulin secretion
- ❏ Explain information related to actions of glucagon
- ❏ Answer questions about control of glucagon secretion
- ❏ Use knowledge of diabetes mellitus
- ❏ Answer questions about pancreatic endocrine-secreting tumors

HORMONES OF THE ISLETS OF LANGERHANS

The location and proportion of each major hormone-secreting cell type of the islets of Langerhans are shown below. The local inhibitory paracrine action of each islet hormone is shown by dashed arrows. The diameter of each circle approximately represents the proportion of that cell type present in the islets.

Clinical Correlate

For many years, C-peptide was considered to have no biological function, but more recently this has been called into question. Studies suggest that C-peptide receptors exist on cells. In addition, C-peptide may serve a protective role, helping to prevent the renal, neural, and microvascular pathologies seen when it is absent, i.e., type I diabetes mellitus.

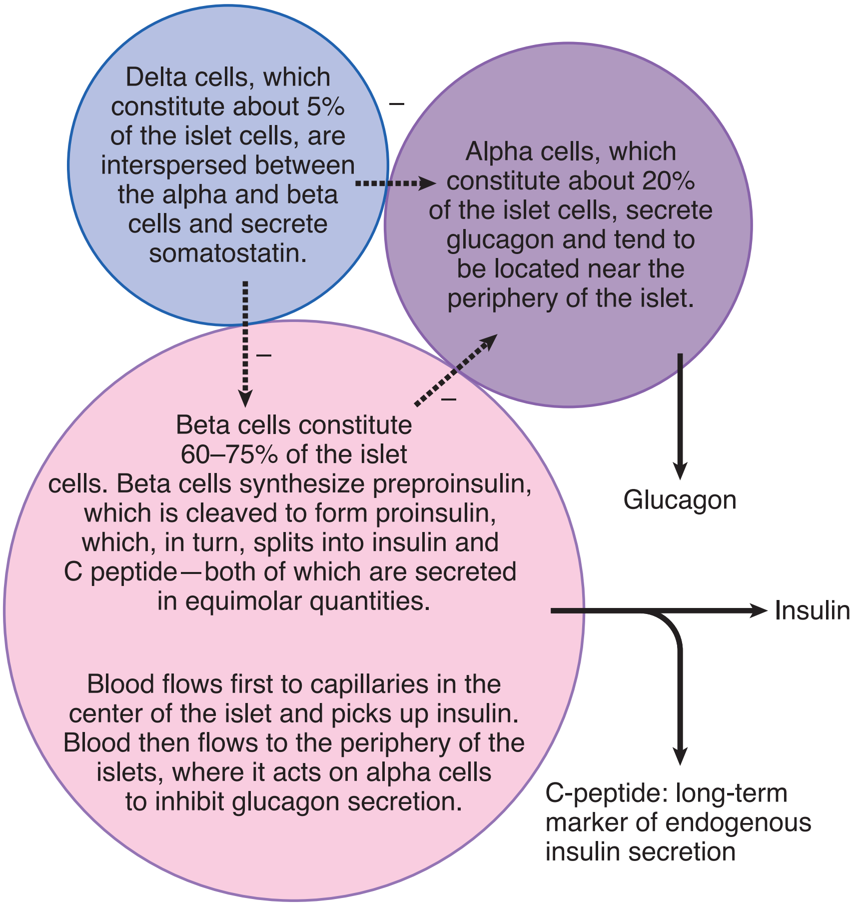


Figure VII-6-1. Hormones of the Pancreatic Islets

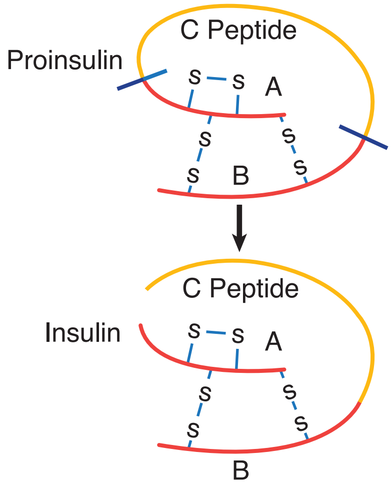


Figure VII-6-2. Insulin

ACTIONS OF INSULIN

Insulin Receptor

The portion of the insulin receptor that faces externally has the **hormone-binding domain**. The portion of the insulin receptor that faces the cytosol has **tyrosine kinase activity**.

When occupied by insulin, the receptor phosphorylates itself and other proteins (*see* Biochemistry Lecture Notes)

Peripheral Uptake of Glucose

Glucose is taken up by peripheral tissues by facilitated diffusion. Insulin facilitates this uptake in some tissues. Typically the insulin receptor causes the insertion of glucose transporters in the membrane.

Tissues that require insulin for effective uptake of glucose are:

- Adipose tissue
- Resting skeletal muscle (although glucose can enter working muscle without the aid of insulin)
- Liver because of glucokinase stimulation

Tissues in which glucose uptake is not affected by insulin are:

- Nervous tissue
- Kidney tubules
- Intestinal mucosa
- Red blood cells
- β-cells of pancreas

Metabolic Actions of Insulin

Insulin is a major anabolic hormone, and it is secreted in response to a carbohydrate- and/or protein-containing meal.

Anabolic hormones tend to promote protein synthesis (increase lean body mass). Other anabolic hormones include:

- Thyroid hormones
- Growth hormone/IGF I
- Sex steroids (androgens)

Effects of insulin on carbohydrate metabolism

Insulin increases the uptake of glucose and its metabolism in muscle and fat. By increasing glucose uptake in muscle, its metabolism increases, i.e., its conversion to carbon dioxide and water is increased.

Insulin increases glycogen synthesis in liver and muscle. The activity of enzymes that promote glycogen synthesis (glucokinase and glycogen synthetase) is increased. The activity of those enzymes that promote glycogen breakdown (phosphorylase and glucose-6-phosphatase) is decreased.

- Glucokinase and glucose-6-phosphatase are expressed by the liver but not by muscle.

Effects of insulin on protein metabolism

- Insulin increases amino acid uptake by muscle cells.
- Insulin increases protein synthesis.
- Insulin decreases protein breakdown (deficiency of insulin results in a breakdown of protein).

Effects of insulin on fat metabolism

Insulin increases:

- Glucose uptake by fat cells (increases membrane transporters). By increasing glucose uptake, insulin also makes triose phosphates available for triglyceride synthesis in adipose tissue.
- Triglyceride uptake by fat cells. It increases the activity of lipoprotein lipase. Lipoprotein lipase is located on the endothelium of capillaries, and it catalyzes the release of free fatty acids from triglycerides.
- Triglyceride synthesis (lipogenesis) in adipose tissue and liver by stimulating the rate-limiting step, namely the carboxylation of acetyl CoA to malonyl CoA. In other words, insulin stimulates the conversion of carbohydrate into fat.

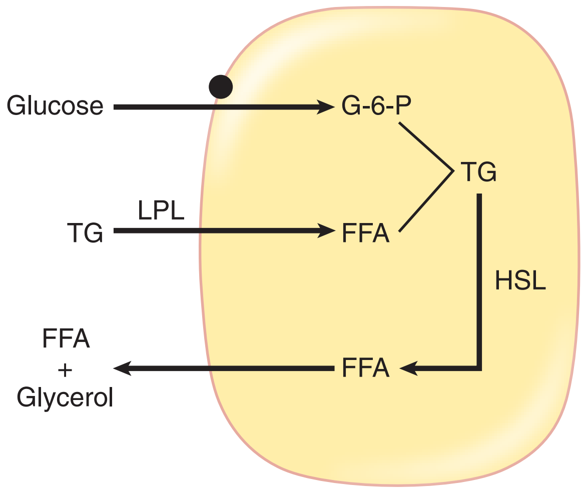


Figure VII-6-3. The Adipose Cell

LPL = Lipoprotein lipase
HSL = Hormone-sensitive lipase

Insulin decreases:

- Triglyceride breakdown (lipolysis) in adipose tissue by decreasing the activity of hormone-sensitive lipase. This enzyme is activated by stress hormones (i.e., cortisol, growth hormone, epinephrine [glucagon]).
- Formation of ketone bodies by the liver.

Insulin Effects on Potassium

Insulin promotes K^+ movement into cells. Although the overall process is not well understood, insulin increases the activity of Na/K-ATPase in most body tissues.

This K^+-lowering action of insulin is used to treat acute, life-threatening hyperkalemia. For example, sometimes the hyperkalemia of renal failure is successfully lowered by the simultaneous administration of insulin and glucose. (The glucose is given to prevent severe insulin-induced hypoglycemia from developing.)

It does not work as quickly as calcium chloride, which is instantaneous, in protecting the heart from arrhythmias. Insulin and glucose administration is faster than Na^+/K^+ cation exchange resins such as Kayexalate. Kayexalate is taken into the GI tract orally but needs 6–12 hours to be effective in lowering potassium levels.

Summary

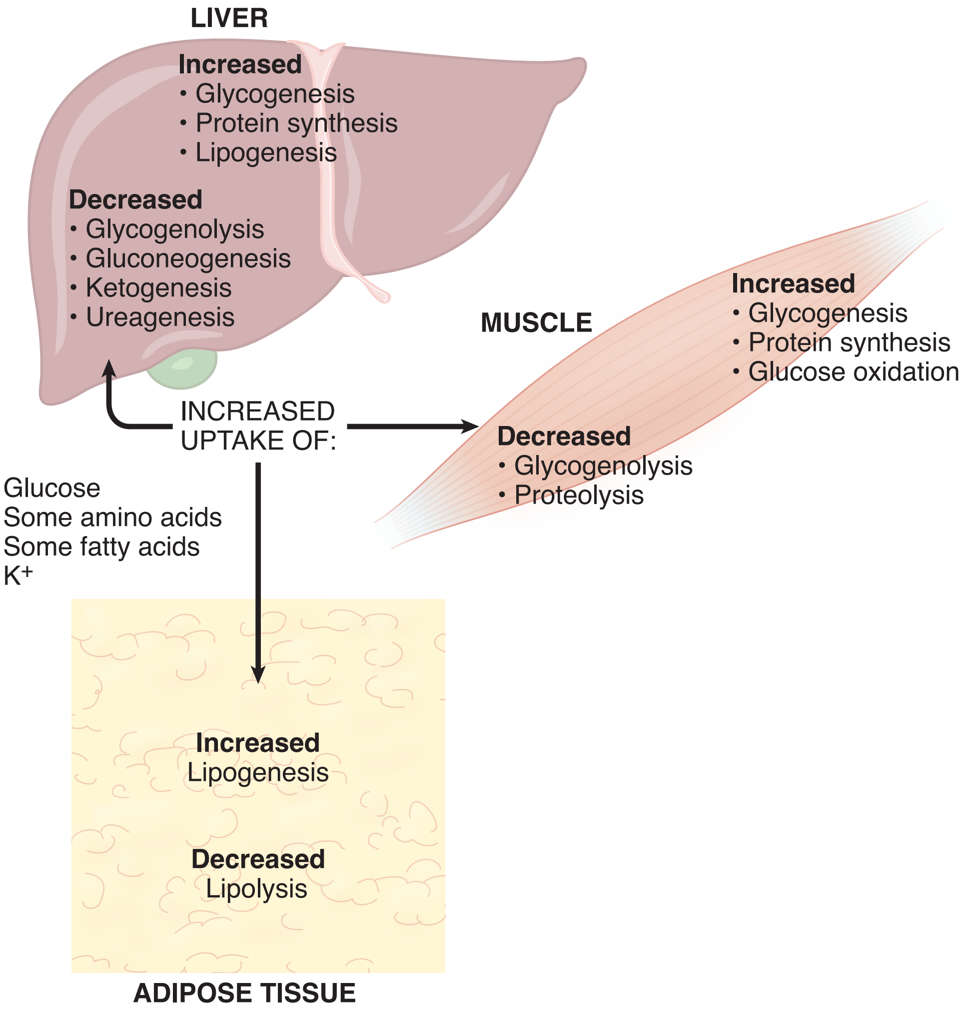


Figure VII-6-4. Major Actions of Insulin

CONTROL OF INSULIN SECRETION

The most important controller of insulin secretion is plasma glucose. Above a threshold of 100 mg%, insulin secretion is directly proportional to plasma glucose.

Glucose enters the cell, causing a rise in intracellular ATP that closes ATP-sensitive K+ channels.

Closure of the ATP-sensitive K^+ channels results in depolarization, causing voltage-gated Ca^{2+} channels to open.

The rise in cytosolic Ca^{2+} causes exocytosis of the vesicles, which then secrete insulin and C-peptide into the blood.

All of the hormones or neurotransmitters named below attach to membrane receptors (R). In contrast, the metabolic substrates, glucose and amino acids, enter the β-cell.

Bridge to Pharmacology

Sulfonylurea derivatives block the ATP-sensitive K^+ channels and thus increase insulin secretion.

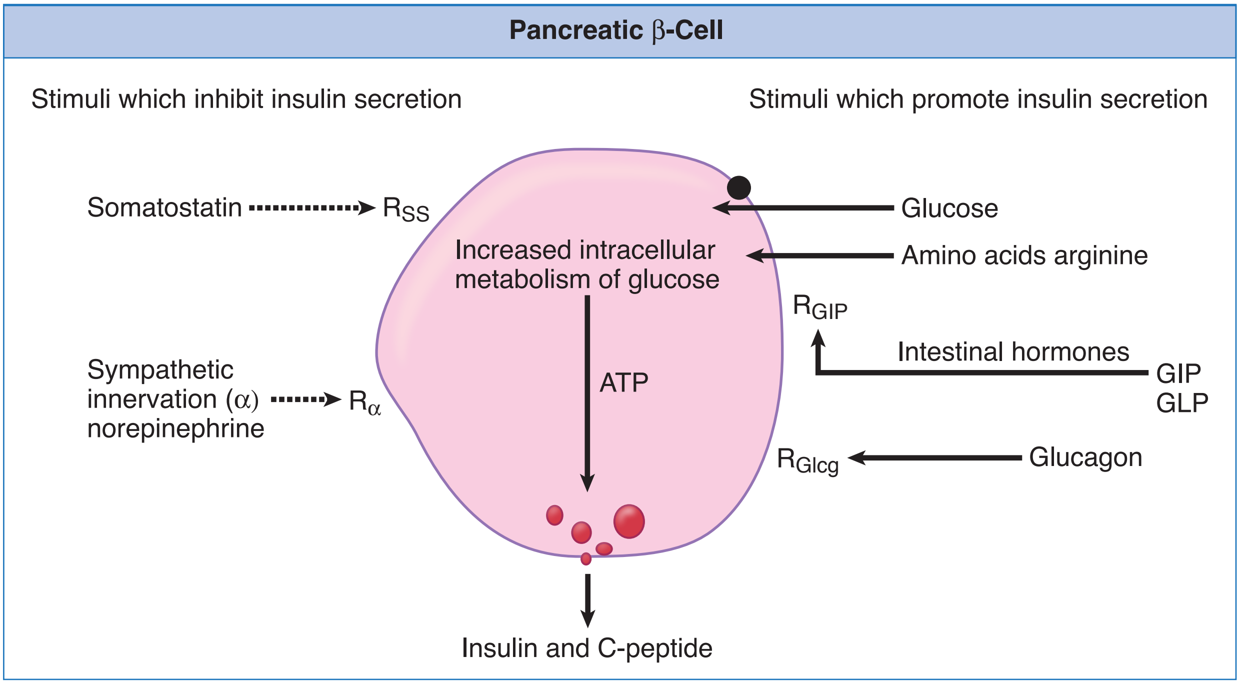


Figure VII-6-5. Control of Insulin Secretion

Incretin

GIP: gastric inhibitory peptide or glucose insulinotropic peptide

GLP: glucagon-like peptide

ACTIONS OF GLUCAGON

Glucagon is a peptide hormone. It is secreted by the α-cells of the pancreatic islets.

The primary target for glucagon action is the liver hepatocyte, where its action is mediated by an increase in the concentration of cAMP. The cAMP activates protein kinase A, which, by catalyzing phosphorylation, alters the activity of enzymes mediating the actions given below.

Note: Skeletal muscle is not a target tissue for glucagon.

Actions of Glucagon on the Liver

There are several specific actions of glucagon on the liver:

- **Increases liver glycogenolysis**
  - Glucagon activates glycogen phosphorylase, breaking down glycogen to glucose-1-phosphate.
  - Glucagon inactivates glycogen synthetase, preventing the glucose-1-phosphate from being recycled back into glycogen.
- **Increases liver gluconeogenesis**
  - Glucagon inhibits phosphofructokinase-2 (PFK-2), thereby reducing 2,6 bisphosphate, which in turn inhibits PFK-1 (an important enzyme driving glycolysis). Inhibition of PFK-1 aids gluconeogenesis.
  - In addition, glucagon, along with cortisol, enhances phosphoenolpyruvate carboxykinase, a key enzyme in the gluconeogenic pathway.
  - Finally, glucagon stimulates glucose-6-phosphatase, thereby releasing glucose into the blood (see Biochemistry Lecture Notes).
- **Increases liver ketogenesis and decreases lipogenesis:** Glucagon inhibits the activity of acetyl CoA carboxylase, decreasing the formation of malonyl CoA. When the concentration of malonyl CoA is low, ketogenesis is favored over lipogenesis.
- **Increases ureagenesis**: It stimulates N-acetylglutamate synthesis, which stimulates the production of urea (see Biochemistry notes).
- **Increases insulin secretion**: The amino acid sequence of glucagon is similar to that of the duodenal hormone, secretin. Like secretin (and most other gut hormones), glucagon stimulates insulin secretion.
- **Increases lipolysis in the liver**: Glucagon activates hormone-sensitive lipase in the liver, but because the action is on the liver and not the adipocyte, glucagon is not considered a major fat-mobilizing hormone.

CONTROL OF GLUCAGON SECRETION

Major factors that control glucagon secretion are summarized in the figure below. Stimuli which **promote** glucagon secretion are depicted on the **right**, and those which **inhibit** are depicted on the **left**.

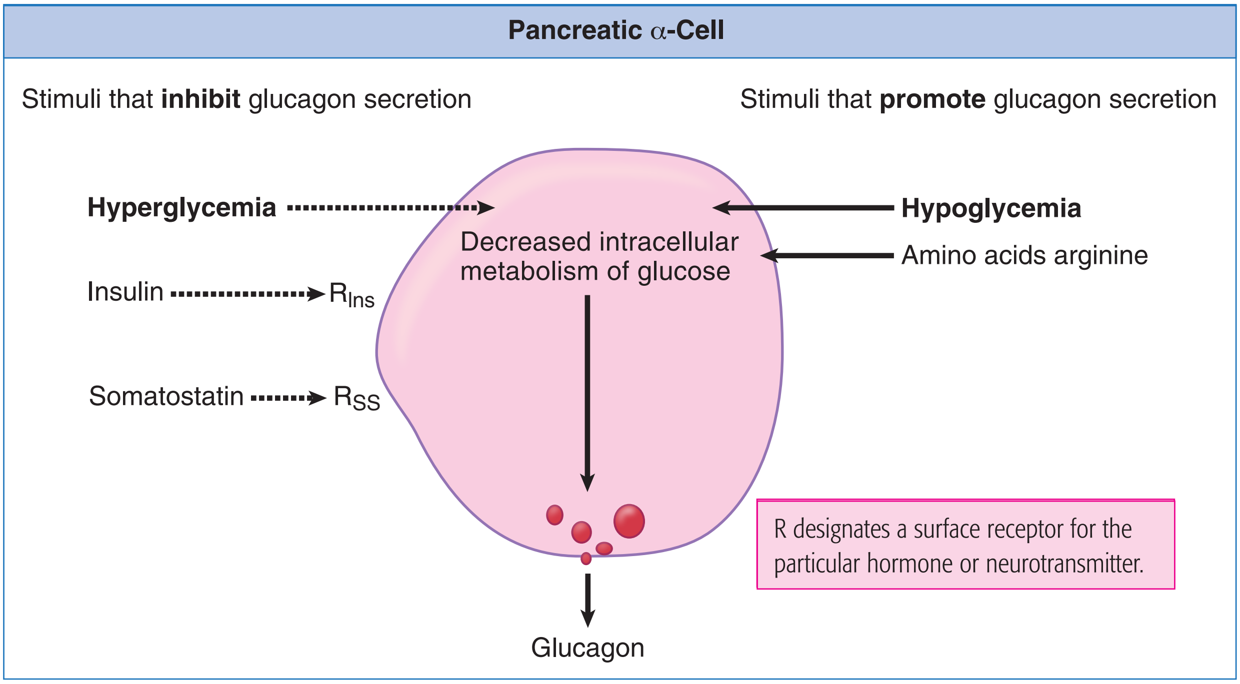


Figure VII-6-6. Control of Glucagon Secretion

Low plasma glucose (hypoglycemia) is the most important physiologic promoter for glucagon secretion, and elevated plasma glucose (hyperglycemia) the most important inhibitor.

Amino acids, especially dibasic amino acids (arginine, lysine), also promote the secretion of glucagon. Thus, glucagon is secreted in response to the ingestion of a meal rich in proteins.

Glucose Counterregulation

Glucose counterregulation is the concept that plasma glucose concentration is regulated by insulin and hormones which oppose (or counter) its actions. The figure below shows glucose regulation in the postprandial and postabsorptive states.

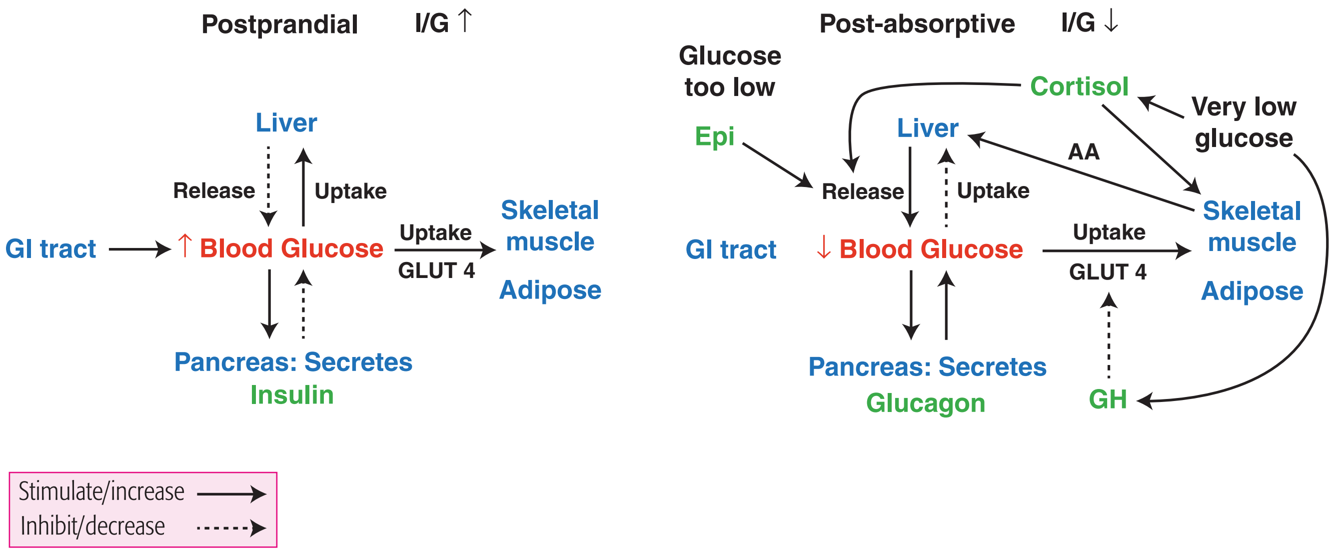


Figure VII-6-7. Insulin Actions in Liver

Insulin : Glucagon Ratio

Insulin and glucagon move substrates in opposite directions. The direction of substrate fluxes is very sensitive to this ratio.

- Normal postabsorptive ratio: 2.0
- States requiring mobilization of substrates ratio: $\leq$0.5
- Carbohydrate meal, ratio $\geq$10
- Protein meal or fat meal produces little change in the ratio

DIABETES MELLITUS

In both types of diabetes mellitus (DM), there is hyperglycemia, polyuria, increased thirst and fluid intake, hyperosmolar state, recurrent blurred vision, mental confusion, lethargy, weakness, and abnormal peripheral sensation.

Coma—if it does occur—is due to the hyperosmotic environment, not the acidosis.

Type 2 DM

Type 2 DM accounts for about 90% of all cases of diabetes. There is a strong genetic component.

- Body build is usually obese (particularly central or visceral).
- Usually middle-aged or older (not always); number of younger patients is increasing
- Characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased hepatic output of glucose. Insulin resistance precedes secretory defects and in the early stages hyperinsulinemia is able to overcome tissue resistance. Ultimately beta cell failure can occur.
- Insulin levels may be high, normal, or low.
- Resistance to insulin is not well understood. It is thought to be due to postreceptor defects in signaling, which ultimately lead to a decrease in the number of glucose transporters. Reducing plasma glucose and thus plasma insulin can increase receptor sensitivity toward normal.
- Plasma glucose is a good screening for type 2. Elevated glucose is due to elevated hepatic output.
- With a controlled diet and exercise, the symptoms of type 2 diabetes often disappear without the necessity for pharmacologic therapy.
- Individuals tend to be ketosis resistant. The presence of some endogenous insulin secretion appears to protect from development of a ketoacidosis. If it does develop, it is usually the result of severe stress or infection (increased counterregulatory hormones, suppressed insulin).
- In nonobese patients, a deficient insulin release by the pancreas is often the problem, but varying degrees of insulin resistance can also occur.

Metabolic Syndrome (Syndrome X)

Metabolic syndrome is a group of metabolic derangements that includes atherogenic dyslipidemia (low HDL) and high triglycerides, elevated blood glucose, hypertension, central obesity, prothrombotic state, and a proinflammatory state.

The clustering of these risk factors increases the probability of developing cardiovascular disease and type 2 DM.

Type 1 DM

The genetic association in type 1 is less marked than in type 2. It affects genetically predisposed individuals whose immune system destroys pancreatic beta cells. Symptoms do not become evident until 80% of the beta cells are destroyed.

- Body build usually lean
- Usually early age of onset (not always)
- Caused by an absence of insulin production
- Increased glucagon secretion also generally occurs
- Three target tissues for insulin—liver, skeletal muscle, and adipose tissue—fail to take up absorbed nutrients (glucose, amino acids, and fatty acids), thus increasing their levels in the blood.

Metabolic effects in insulin-deficient individuals

CHO

- Increased blood glucose concentration
- Increased glycogen breakdown
- Decreased peripheral glucose use

Protein

- Increased protein breakdown
- Increased catabolism of amino acids
- Increased gluconeogenesis
- Increased ureagenesis
- Decreased protein synthesis

Fat

- Increased triglyceride breakdown
- Increased level of circulating free fatty acids
- Increased ketosis, resulting in ketoacidosis (metabolic acidosis)
- Decreased fatty acid synthesis
- Decreased triglyceride synthesis

Renal System

The failure to reabsorb all the filtered glucose in the proximal tube also prevents normal water and electrolyte reabsorption in this segment, resulting in an osmotic diuresis (polyuria). This causes loss of glucose, water, and electrolytes from the body. Thus, even though the electrolyte concentration of the urine is low, body stores of electrolytes, particularly Na^+ and K^+, are lost.

Potassium Ion

- Hydrogen ions move intracellularly to be buffered, and potassium ions leave the cell, reducing the intracellular concentration.
- There is a lack of the normal insulin effect of pumping potassium ion into cells.
- Consequently, hyperkalemia is typical, but plasma K^+ may be normal or low because of renal loss. Regardless, the body stores of K^+ are reduced because of the renal loss.
- Insulin replacement can produce severe hypokalemia, and potassium replacement is a normal part of therapy.

Sodium Ion

- Polyuria decreases total body sodium but dehydration may keep sodium within or close to the normal range.
- Hyperosmolar state due to the hyperglycemia. Thus, 2 times the sodium concentration is not a good index of osmolarity.

$$\text{Effective osmolarity} = 2\ (\text{Na})\ \text{mEq/L} + \frac{\text{glucose mg/dL}}{18}$$

Hyperosmolar Coma

- Severe hyperglycemia shifts fluid from the intracellular to the extracellular space.
- Polyuria decreases volume of the extracellular space and leads to a decreased renal plasma flow and a reduced glucose excretion. Combined with the rise in counterregulatory hormones, the plasma glucose rises further.
- The severe loss of intracellular fluid from the brain causes the coma.
- Type 2 diabetics often present with the highest plasma glucose and greater states of dehydration. Thus these patients have a higher incidence of coma.

Diabetic Ketoacidosis (DKA)

Without any insulin, excessive lipolysis provides fatty acids to the liver, where they are preferentially converted to ketone bodies because of the unopposed action of glucagon.

- Blood pH and bicarbonate decrease due to the metabolic acidosis.
- Increased alveolar ventilation is the respiratory compensation for the metabolic acidosis. When the arterial pH decreases to about 7.20, ventilation becomes deep and rapid (Kussmaul breathing).
- An acidic urine results as the kidneys attempt to compensate for the acidosis.
- The severe acidosis is in addition to the dehydration and net decrease in total body sodium and potassium.
- Treatment is replacement of fluid and electrolytes and administration of insulin
- DKA treatment is first 2–3 liters of normal saline and IV insulin. Subcutaneous insulin may not be fully absorbed because of decreased skin perfusion. Hyponatremia is common because of hyperglycemia. For each 100 point increase in glucose above normal, there is a 1.6 decrease in sodium.

  100 mg ELEVATION glucose = 1.6 mEq DECREASE sodium

  When hyponatremia is present with hyperglycemia, management is correction of the elevated glucose level. When glucose comes to normal, the sodium corrects.

Hypoglycemia

In the diabetic, overdosing with insulin causes hypoglycemia. Type 1 diabetics are particularly prone to hypoglycemia; in these individuals the glucagon response to hypoglycemia is absent.

Initial symptoms due to catecholamine release followed by the direct effects of hypoglycemia include slowed mental processes and confusion.

PANCREATIC ENDOCRINE-SECRETING TUMORS

Insulinomas

Insulinomas are the most common islet cell tumor. They are found almost exclusively within the pancreas, and they hypersecrete insulin.

- Most common symptoms due to the hypoglycemia (confusion, disorientation, headache)
- Association with MEN 1
- Insulin measured to determine insulin-mediated versus noninsulin-mediated hypoglycemia
- Insulin-secreting tumor: insulin and C-peptide both elevated
- Factitious hypoglycemia: C-peptide below normal
- Treat with removal

Other Endocrine-Secreting Tumors

- Gastrinomas
- Glucagonomas
- Somostatinomas
- VIPomas

Management of all neuroendocrine tumors is localization with CT, then surgical resection.

Glucagonoma

- Alpha cell oversecretion
- Hyperglycemia/diabetes
- Localize with CT scan
- Surgically remove

Summary

Table VII-6-1. Insulin-Related Pathophysiologic States

	Glucose	Insulin	C-peptide	Ketoacidosis
Type 2 diabetes	↑	↑, ↔	↑, ↔	–
Type 1 diabetes	↑	↓	↓	+
Insulinoma	↓	↑	↑	–
Factitious hypoglycemia (self-injection of insulin)	↓	↑	↓	–

OTHER HORMONES INVOLVED IN ENERGY BALANCE AND APPETITE

Leptin

Leptin is produced in adipose tissue and is thought to be a "long-term" regulator of appetite and energy balance. Secretion is circadian, with the highest levels occurring at night and the nadir in the morning. Individual meals do not stimulate the release of leptin.

- The leptin receptor is a member of the cytokine family of receptors, which activate gene transcription factors.
- Leptin decreases hypothalamic neuropeptide Y (NPY), which is a potent activator of feeding (orexigenic). By inhibiting NPY synthesis, leptin promotes satiety (anorexigenic).
- Leptin increases energy expenditure, in part by increasing fatty acid oxidation, and it decreases fat stores. Lack of and/or resistance to leptin causes obesity.

Adiponectin

Adiponectin is produced in adipose tissue, and it increases insulin sensitivity and tissue fat oxidation.

- Dysregulation of adiponectin, along with production of cytokines by adipocytes, may play a role in obesity, insulin resistance, and cachexia. Plasma levels of adiponectin are low in type 2 diabetics, and infusion of this hormone decreases plasma glucose in experimental animal models of DM.
- The mechanism of action and regulation of secretion are not well understood, but it does appear to inhibit liver output of glucose.

Bridge to Pharmacology

Administration of the thiazolidinedione (TZD) class of compounds to diabetics increases the circulating levels of adiponectin, which may be part of the mechanism by which these drugs reduce plasma glucose.

Ghrelin

Ghrelin is produced by cells of the stomach. Circulating levels of ghrelin are reduced in response to a meal and highest in the fasting state.

- Ghrelin activates hypothalamic NYP neurons (see leptin discussion above) and is thus a potent orexigenic hormone. It also stimulates the release of growth hormone (GH), although its physiologic significance/role is not well understood. Because of ghrelin's effects on GH and appetite, however, it may prove beneficial for restoring GH levels in the elderly and anorexic conditions, such as cancer.
- Ghrelin levels are decreased in obese individuals and elevated by low calorie diets, strenuous exercise, and patients with Prader-Willi syndrome.
- Ghrelin is a peptide hormone that works via Gq and Gs. Its mechanism of action and regulation of secretion are not well understood.

Bridge to Pathology

Prader-Willi syndrome is a genetic condition affecting many parts of the body. In infancy, it is characterized by hypotonia, feeding difficulties, poor growth, and delayed development. In childhood, patients develop an insatiable appetite, leading to chronic hyperphagia and obesity.

Recall Question

Which of the following lab values is most consistent with sulfonylurea abuse?

A. Increased serum glucose, increased insulin, increased C-peptide

B. Increased serum glucose, decreased insulin, decreased C-peptide

C. Decreased serum glucose, increased serum insulin, decreased serum C-peptide

D. Decreased serum glucose, increased serum insulin, increased serum C-peptide

E. Increased serum glucose, increased serum insulin, decreased serum C-peptide

Answer: D

Hormonal Control of Calcium and Phosphate

7

Learning Objectives

- ❑ Solve problems concerning overview of calcium and phosphate
- ❑ Solve problems concerning bone remodeling
- ❑ Solve problems concerning parathyroid hormone
- ❑ Solve problems concerning calcitonin
- ❑ Demonstrate understanding of role of vitamin D (calcitriol) in calcium homeostasis
- ❑ Solve problems concerning disorders in calcium and phosphate
- ❑ Answer questions about metabolic bone disorders

CALCIUM AND PHOSPHATE

- The percentage of dietary calcium absorbed from the gut is inversely related to intake.
- The dietary intake of and the percentage of calcium absorbed is diminished in the elderly.
- Ingested phosphate is also absorbed by the gut.
- Both calcium and phosphate absorption in the GI tract are stimulated by the active form of vitamin D (calcitriol).

The approximate percentage of the body's total calcium is given for each of the compartments in the figure below. In addition, the fraction of calcium is indicated. The calcium concentration in the interstitial fluid is 103–104 times higher than the intracellular calcium concentration. The initiation of many cellular processes (secretion, movement of intracellular organelles, cell division) is linked to a sudden brief increase in intracellular (cytosolic) calcium.

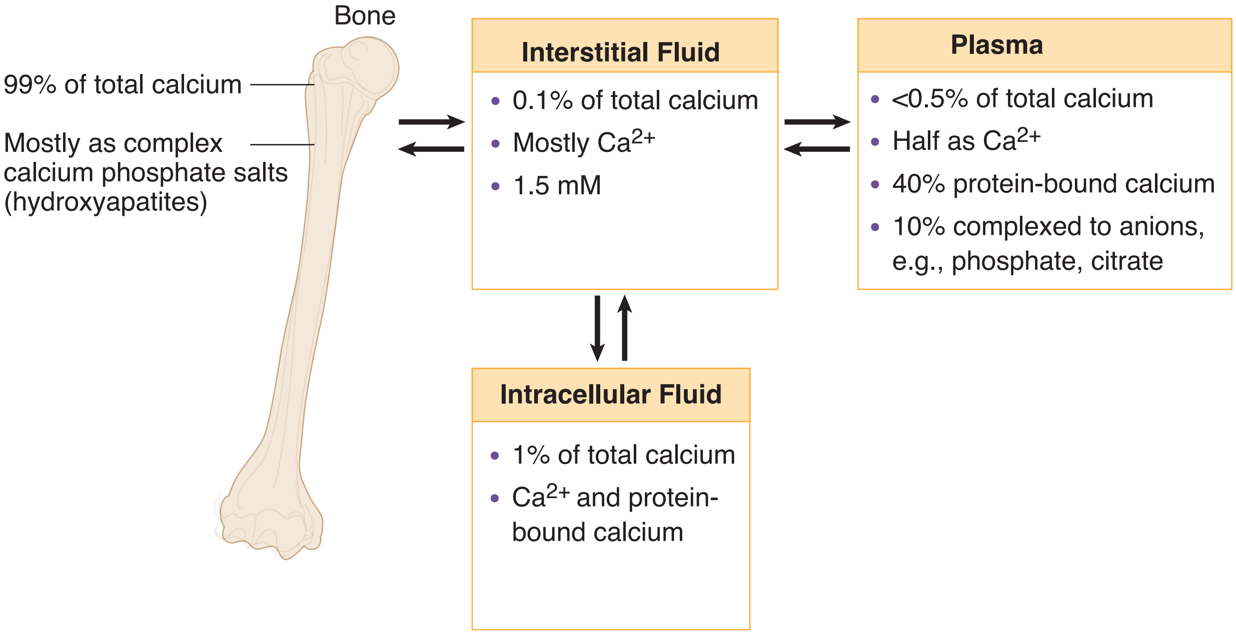


Figure VII-7-1. Calcium Distribution in the Body

Plasma Calcium

Plasma calcium represents 50% ionized free, 40% attached to protein, and 10% associated with anions such as phosphate and citrate. The free calcium is the physiologically active and precisely regulated form.

- Alkalosis (hyperventilation) decreases and acidosis increases free plasma calcium by varying the amount bound to protein.
- Alkalosis lowers free calcium by increasing protein-binding, while acidosis raises free calcium by decreasing protein-binding.

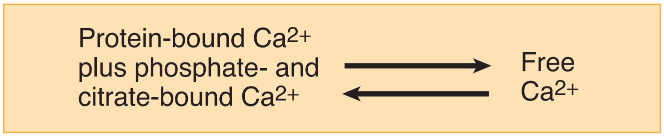


Figure VII-7-2. Relationship of Bound and Free Calcium

Relationship Between Calcium and Phosphate

Bone is a complex precipitate of calcium and phosphate to which hydroxide and bicarbonate ions are added to make up the mature hydroxyapatite crystals that are laid down in a protein (osteoid) matrix.

Whether calcium and phosphate are laid down in bone (precipitate from solution) or are resorbed from bone (go into solution) depends on the product of their concentrations rather than on their individual concentrations.

When the product exceeds a certain number (solubility product or ion product), bone is laid down:

$$[Ca^{2+}] \times [PO^{-}{}_4] > \text{solubility product} = \text{bone deposition}$$

- Under normal conditions the ECF product of calcium times phosphate is close to the solubility product.
- Thus, an increase in the interstitial fluid concentration of Ca^{2+} or phosphate will increase bone mineralization.
- For example, an increase in plasma phosphate would increase the product of their concentrations, promote precipitation, and lower free calcium in the interstitial fluid.
- A malignant increase in the concentration of calcium or phosphate due to chronic renal disease or rhabdomyolysis can cause the precipitation of calcium phosphate within tissues.

When the product is below the solubility product, bone is resorbed:

$$[Ca^{2+}] \times [PO^{-}{}_4] < \text{solubility product} = \text{bone resorption}$$

- Thus, a decrease in the interstitial concentration of Ca^{2+} or phosphate will promote the resorption of these salts from bone (demineralization).
- For example, a decrease in plasma phosphate alone would promote bone demineralization. Increasing renal excretion of phosphate would promote bone demineralization and a rise in interstitial free calcium.

It is the free Ca^{2+}— not the phosphate—that is regulated so precisely. Hormonal control of free Ca^{2+} levels is via a dual hormonal system: parathyroid hormone and vitamin D.

BONE REMODELING

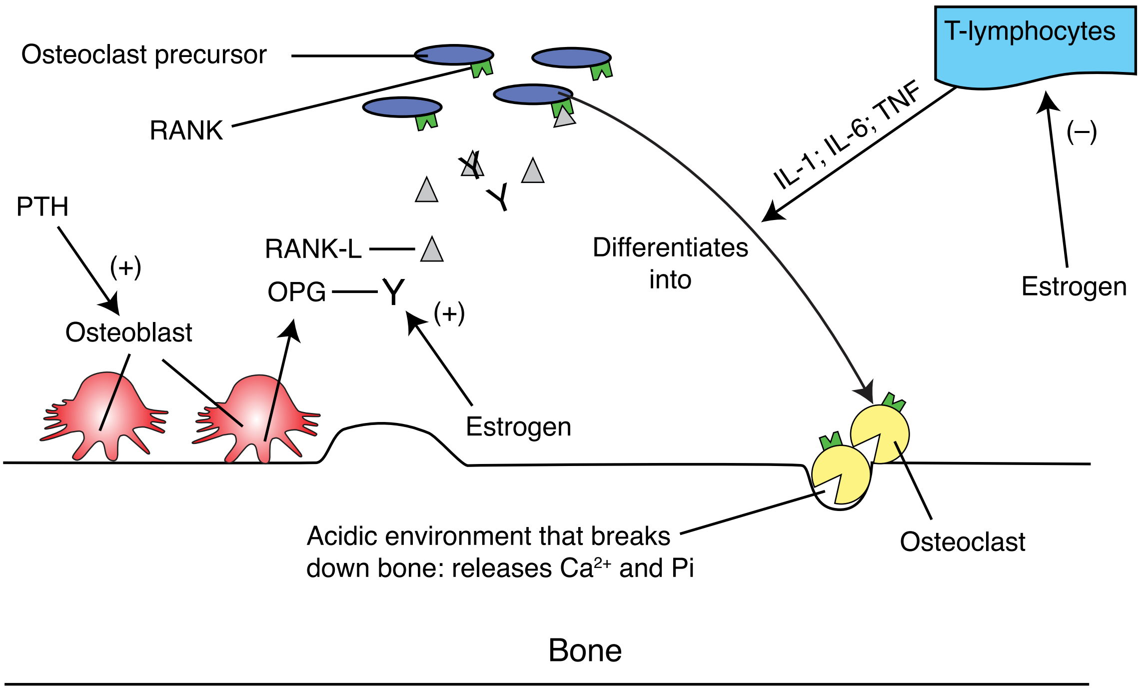
Bone undergoes continual remodeling throughout life, although the turnover is faster in younger individuals. Bone remodeling involves the interplay between bone-building cells (osteoblasts) and cells that break down bone (osteoclasts).

- Osteoblasts cause bone deposition; they secrete 2 proteins:
  - **RANK-L** (Receptor Activator of Nuclear KappaB Ligand) binds to the RANK receptor, which is expressed on precursor cells resulting in their differentiation into active osteoclasts. Active osteoclasts also express the RANK receptor, which, when stimulated, activates osteoclastic activity.
  - **OPG** (osteoprotegerin) binds RANK-L, thereby preventing it from binding onto precursor or osteoclast cells. This reduces differentiation and overall osteoclastic activity. Thus, OPG acts as a “decoy” for RANK-L.

Note

As many as 300,000 bone-remodeling sites are active in a normal person.

- Bone remodeling is influenced by parathyroid hormone (PTH), and the active form of vitamin D. Estrogen is well known for conserving bone integrity and it does so by at least 2 mechanisms:
  - Induces the synthesis of OPG.
  - Reduces the secretion of cytokines by T-lymphocytes; these cytokines stimulate differentiation of precursor cells into active osteoclasts and they stimulate activity of mature osteocytes
  - By inhibiting cytokines and increasing OPG, estrogen reduces the activity of osteoclasts.
- Glucocorticoids increase bone breakdown by inducing the synthesis and release of RANK-L and by inhibiting the synthesis of OPG.



RANK = receptor activator of nuclear factor kappaB

RANK-L = receptor activator of nuclear factor kappaB ligand

OPG = osteoprotegerin (endogenous blocker of RANK-L)

Pi = phosphate

Figure VII-7-3. Relationship between Osteoblasts and Osteoclasts

Weight-Bearing Stress

Weight-bearing mechanical stress increases the mineralization of bone. The absence of weight-bearing stress (being sedentary, bedridden, or weightless) promotes the demineralization of bone. Under these conditions, the following occurs:

- Plasma Ca^{2+} tends to be in the upper region of normal.
- Plasma PTH decreases.
- Urinary calcium increases.

Indices

Indices can be utilized to detect excess bone demineralization and remodeling:

- Increased serum osteocalcin and alkaline phosphatase are associated with osteoblastic activity.
- Increased urinary excretion of hydroxyproline is a breakdown product of collagen

PARATHYROID HORMONE (PTH)

Actions of PTH

A decrease in the free calcium is the signal to increase PTH secretion and the function of PTH is to raise free calcium, which it does by several mechanisms.

- Increases Ca^{2+} reabsorption in distal tubule of the kidney (*see* chapter 4, Part VI)
- Inhibits phosphate (Pi) reabsorption in proximal tubule of the kidney.
- Stimulates the 1-alpha-hydroxylase enzyme in kidney, converting inactive vitamin D to its active form.
- Causes bone resorption, releasing Ca^{2+} and Pi into the blood.

Bone resorption

The mechanisms of PTH-induced bone resorption are complex and not fully understood. However, the following generalizations do apply.

- Osteoblasts express receptors for PTH. Binding of PTH stimulates the osteoblast to release RANK-L. This in turn, increases osteoclastic activity resulting in bone resorption and the release of calcium and phosphate into the blood.
- Although counterintuitive, intermittent spikes in PTH, e.g., intravenous or subcutaneous injection, stimulates osteoblastic activity resulting in bone deposition. Thus, PTH can be useful in treating osteoporosis in the clinical setting.

Parathyroid Hormone-Related Peptide

Parathyroid hormone-related peptide (PTHrP) is a paracrine factor secreted by many tissues; e.g., lung, mammary tissue, placenta. It may have a role in fetal development. In postnatal life, its role is unclear.

- The majority of humoral hypercalcemias of malignancy are due to overexpression of PTHrP.
- PTHrP has a strong structural homology to PTH and binds with equal affinity to the PTH receptor.

Regulation of PTH Secretion

PTH is a peptide hormone released from the parathyroid glands in response to lowered plasma free Ca^{2+}. Free Ca^{2+} in the plasma is the primary regulator of PTH.

- The negative feedback relationship between plasma calcium and PTH secretion is highly sigmoidal, with the steep portion of the curve representing the normal range of plasma free calcium.
- To sense the free calcium, the parathyroid cell depends upon high levels of expression of the calcium-sensing receptor (CaSR).
- In most cells, exocytosis depends on a rise in intracellular free calcium. In the parathyroid gland, a fall in intracellular free calcium causes release.
- Depletion of magnesium stores can create a reversible hypoparathyroidism.

Normal range = region between dashed lines

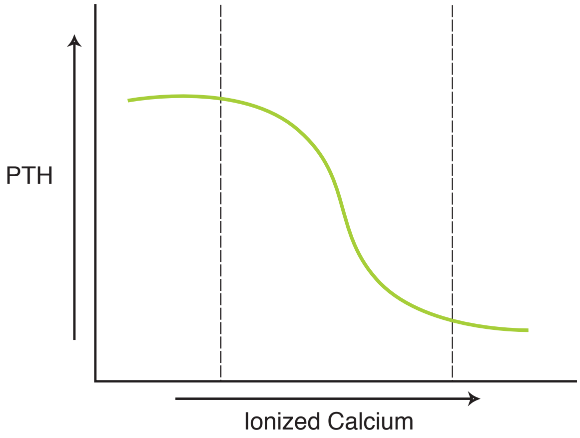


Figure VII-7-4. Relationship between Plasma Calcium and PTH

CALCITONIN

Calcitonin is a peptide hormone secreted by the parafollicular cells (C cells) of the thyroid gland. It is released in response to elevated free calcium.

- Calcitonin lowers plasma calcium by decreasing the activity of osteoclasts, thus decreasing bone resorption. It is useful in the treatment of Paget's disease, severe hypercalcemia, and osteoporosis.
- Calcitonin is not a major controller of Ca^{2+} in humans. Removing the thyroid (with the C cells) or excess of calcitonin via a C cell tumor (medullary carcinoma of the thyroid) has little impact on plasma calcium.
- No deficiency or excess disease has been described.

ROLE OF VITAMIN D (CALCITRIOL) IN CALCIUM HOMEOSTASIS

Sources and Synthesis

Vitamin D_2 (ergocalciferol) is a vitamin but can functionally be considered a prohormone. It is a normal dietary component. A slightly different form, vitamin D_3 (cholecalciferol), is synthesized in the skin. The synthesis of active 1,25 di-OH vitamin D (calcitriol) is outlined below.

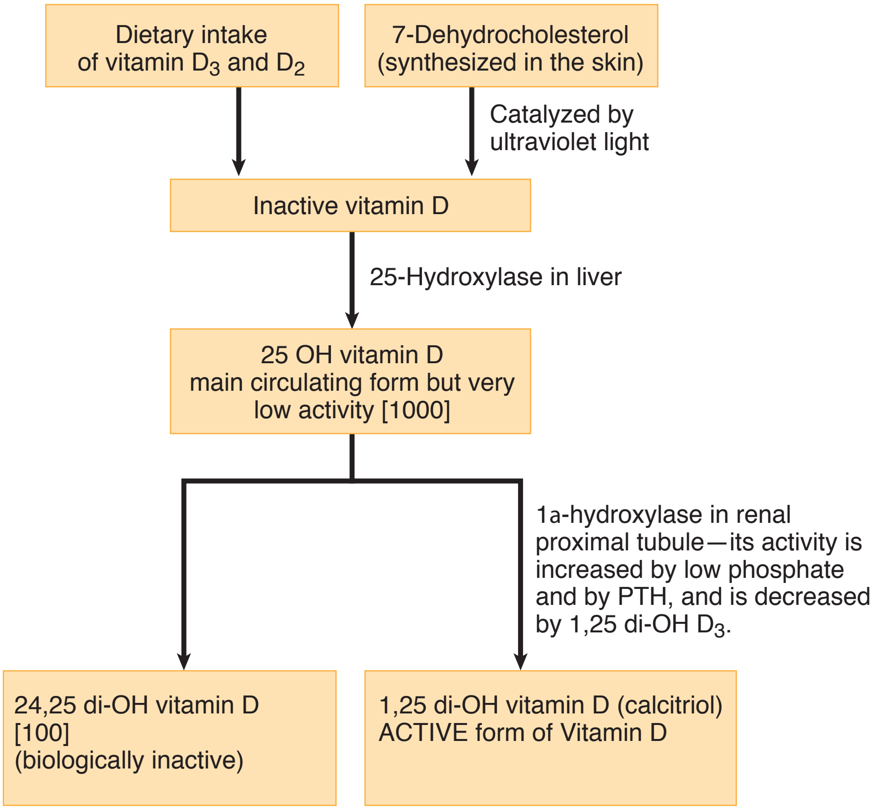


Figure VII-7-5. Vitamin D Metabolism

The synthesis of calcitriol occurs sequentially in the skin → liver → kidney. The relative numbers of molecules of each of the hydroxylated forms of D present in the blood of a normal person are given in brackets. After its conversion to the 25 OH form in the liver, it can be stored in fat tissue. The serum levels of 25 OH vitamin D represent the best measure of the body stores of vitamin D when a deficiency is suspected. Most of the 25 OH form, which is the immediate precursor of 1,25 di-OH D, is converted to the inactive metabolite, 24,25 di-OH D. Ultraviolet (UV) light also evokes skin tanning, decreasing the penetration of UV light, and thus decreases the subsequent formation of D_3. This mechanism may prevent overproduction of D_3 in individuals exposed to large amounts of sunlight.

Actions of Calcitriol

Under normal conditions, vitamin D acts to raise plasma Ca^{2+} and phosphate. Thus, vitamin D promotes bone deposition. This is accomplished by:

- Calcitriol increases the absorption of Ca^{2+} and phosphate by the intestinal mucosa by increasing the production of the Ca^{2+}-binding protein calbindin. The details of this process are poorly understood.
- The resulting high concentrations of Ca^{2+} and phosphate in the extracellular fluid exceed the solubility product, and precipitation of bone salts into bone matrix occurs.
- Calcitriol enhances PTH's action at the renal distal tubule.

At abnormally high activity levels calcitriol increases bone resorption and release of Ca^{2+} and phosphate from bone. Receptors for calcitriol are on the nuclear membranes of osteoblasts. Through communication from osteoblasts, activated osteoclasts carry out the bone resorption. Calcitriol requires the concurrent presence of PTH for its bone-resorbing action.

The figure below provides an overview of regulation of calcium and phosphate by parathyroid hormone and vitamin D.

Summary

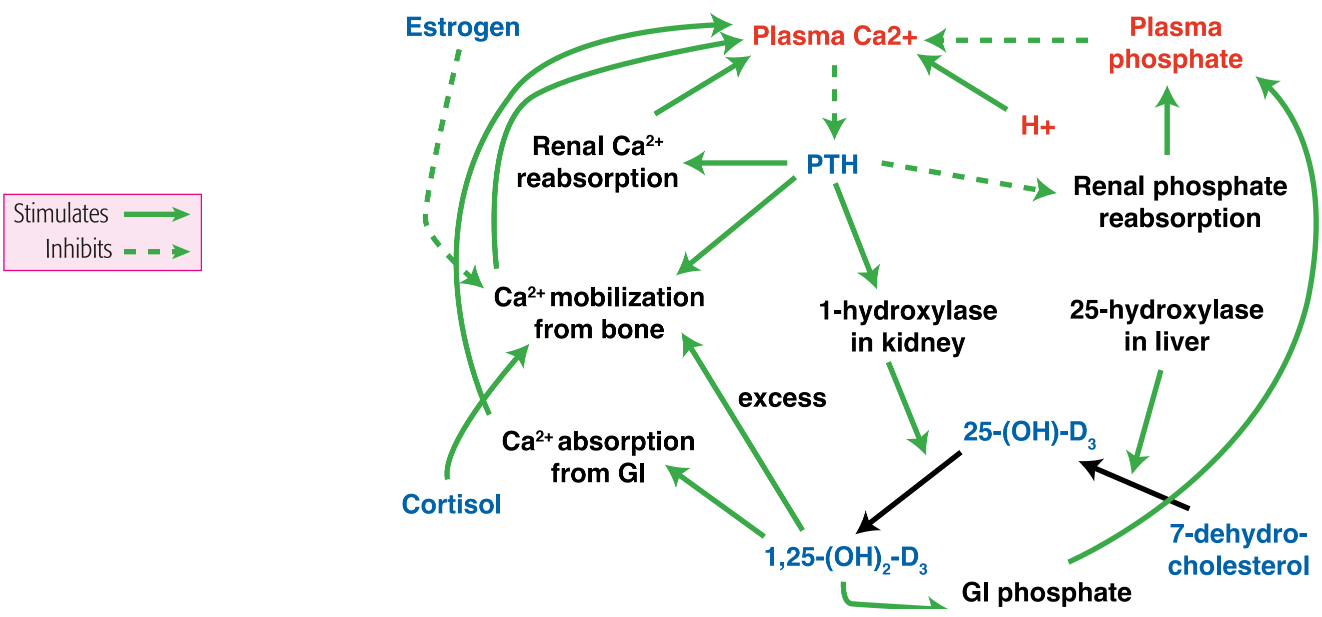


Figure VII-7-6. Regulation of Calcium and Phosphate

DISORDERS IN CALCIUM AND PHOSPHATE

Hypercalcemia

Hypercalcemia of primary hyperparathyroidism

- Initiating factor is primary hypersecretion of PTH
- Consequences include increased plasma calcium, decreased plasma phosphate, polyuria, hypercalciuria, and decreased bone mass
- 80% due to a single parathyroid adenoma
- High calcium can lead to nephrogenic diabetes insipidus. This is why there is massive volume deficit in hypercalcemia.
- High calcium makes it harder to depolarize neural tissue. This is why hypercalcemia causes lethargy, confusion, and constipation.
- 15% due to primary hyperplasia as in MEN 1 or MEN 2A
- Parathyroid carcinoma rare

- Ectopic hormonal hypercalcemia usually PTHrP
- Most patients asymptomatic
- Symptoms include lethargy, fatigue, depression, neuromuscular weakness, and difficulty in concentrating
- Increased plasma alkaline phosphatase, osteocalcin and increased excretion of cAMP (second messenger for PTH in the kidney), and hydroxyproline
- Severe dehydration
- Bone manifestation is osteitis fibrosa cystica in which there are increased osteoclasts in scalloped areas of the surface bone and replacement of marrow elements with fibrous tissue. Increased alkaline phosphatase is due to high bone turnover.
- Hypercalcemia decreases QT interval and in some cases causes cardiac arrhythmias.

Related causes of hypercalcemia

- Lithium shifts the sigmoid Ca/PTH curve to the right. Higher calcium levels are thus needed to suppress PTH. Similarly, the CaSR is mutated in patients with familial hypocalciuric hypercalcemia (FHH; see Renal Physiology section, chapter 4), resulting in more PTH for any given calcium concentration in the plasma.
- Sarcoidosis and other granulomatous disorders (10%) due to increased activity of 1-alpha hydroxylase activity in granulomas.
- Thyrotoxicosis, milk-alkali syndrome
- Thiazide diuretics increase renal calcium absorption.

Differential diagnosis and treatment

- Elevated plasma calcium and PTH normal or elevated; conclusion is primary hyperparathyroidism
- Elevated plasma calcium and decreased PTH; conclusion is something other than primary hyperparathyroidism
- Treatment is usually surgery; i.e., removing the adenoma or with hyperplasia removing most of the parathyroid tissue
- Treat with high volume fluid replacement
- Bisphosphonates need 2–3 days to be fully effective
- Calcitonin rapidly inhibits osteoclastic activity

Hypocalcemia

Hypocalcemia of primary hypoparathyroidism

- Can be hereditary or autoimmune
- Caused by thyroid surgery or surgery to correct hyperparathyroidism
- The initiating factor is inadequate secretion of PTH by the parathyroid glands.

- The decrease in plasma calcium is accompanied by an increased plasma phosphate. Even though less phosphate is resorbed from bone, plasma phosphate increases because the normal action of PTH is to inhibit phosphate reabsorption and increase excretion by the kidney. Therefore, without PTH, more of the filtered load is reabsorbed.
- Symptoms focus on the hypocalcemic induced increased excitability of motor neurons creating muscular spasms and tetany
- Chvostek's sign is induced by tapping the facial nerve just anterior to the ear lobe.
- Trousseau's sign is elicited by inflating a pressure cuff on the upper arm. A positive response is carpal spasm.
- Hypomagnesemia prevents PTH secretion and induces hypocalcemia. This condition responds immediately to an infusion of magnesium.

Pseudohypoparathyroidism

- This is a rare familial disorder characterized by target tissue resistance to parathyroid hormone.
- Exhibits same signs and symptoms as primary hypoparathyroidism except PTH elevated
- It is usually accompanied by developmental defects: mental retardation, short and stocky stature, one or more metacarpal or metatarsal bones missing (short 4th or 5th finger).

Additional causes of hypocalcemia

- Acute hypocalcemia can occur even with intact homeostatic mechanisms. Included would be alkalosis via hyperventilation, transfusions of citrated blood, rhabdomyolysis or tumor lysis, and the subsequent hyperphosphatemia
- Hyperphosphatemia of chronic renal failure
- Failures with vitamin D system
- Congenital absence of parathyroids rare (DiGeorge's syndrome)
- Damaged muscle binds calcium. Rhabdomyolysis binds free calcium.

Predictive indices for a primary disorder

When plasma calcium and phosphate levels are changing in opposite directions, the cause is usually a primary disorder. An exception may be chronic renal failure. This state is not a primary disorder but is usually associated with hypocalcemia and hyperphosphatemia (hypocalcemic-induced secondary hyperparathyroidism).

Renal Failure and Secondary Hyperparathyroidism

- Most common cause of secondary hyperparathyroidism
- Loss of nephrons prevents kidneys from excreting phosphate (Pi)
- Elevated Pi lowers free Ca^{2+}, which in turn increases PTH

Vitamin D Deficiency and Secondary Hyperparathyroidism

- Causes include a diet deficient in vitamin D, inadequate sunlight exposure, malabsorption of vitamin D, enzyme deficiencies in the pathway to activation of vitamin D
- In all cases there is a decrease in plasma calcium, which elicits an increase in PTH secretion and a secondary hyperparathyroidism.
- A similar consequence is the increased demand for calcium as in pregnancy.
- Characterized by increased PTH, decreased plasma calcium, and decreased plasma phosphate. Even though the elevated PTH increases phosphate resorption from bone, PTH also inhibits phosphate reabsorption by the kidney, thereby promoting phosphate excretion and a drop in plasma phosphate.
- Bone mass is lost to maintain plasma calcium.
- Diagnostic test is a low plasma 25(OH) vitamin D.

Excess Vitamin D and Secondary Hypoparathyroidism

- An excessive intake of vitamin D raises plasma calcium, which elicits a decrease in PTH
- Characterized by decreased PTH, increased plasma calcium, and increased plasma phosphate but normal or decreased phosphate excretion. Because PTH increases the excretion of phosphate by inhibiting reabsorption in the proximal tubule, decreased PTH causes increased reabsorption of phosphate and elevated plasma levels.
- Excessive vitamin D promotes bone resorption and bone mass decreases.

Predictive indices for a secondary disorder

When the plasma calcium and phosphate are changing in the same direction, the origin is usually a secondary disorder.

- Secondary **hyper**parathyroidism: both decrease
- Secondary **hypo**parathyroidism: both increase

Note also that with either a deficiency or an excess of vitamin D, there is a decrease in bone mass but mechanism differs (high PTH in deficiency; direct effect of vitamin D with excess).

Recall Question

Estrogen conserves bone integrity by which of the following mechanisms?

A. Induces the synthesis of RANK-L receptors

B. Reduces the activity of osteoblasts

C. Reduces the secretion of cytokines by T-lymphocytes

D. Increases differentiation and overall osteoclastic activity

E. Increases bone breakdown

Answer: C

METABOLIC BONE DISORDERS

Osteoporosis

Osteoporosis is a loss of bone mass (both mineralization and matrix) with fractures, caused by normal age-related changes in bone remodeling and other factors that exaggerate this process.

- If bone mineral density is 2.5 standard deviations below the average, that equals osteoporosis.
- If bone mineral density is 1–2.5 standard deviations below the average, that equals osteopenia.
- Bone mass reaches a peak subsequent to puberty. Heredity accounts for most of the variation but physical activity, nutrition, and reproductive hormones play a significant role, especially estrogens (even in men).
- Secondary osteoporosis can occur in thyrotoxicosis and particularly with elevations in glucocorticoids.
- Calcitonin inhibits bone resorption.

A mainstay of treatment is bisphosphonates, which are rapidly incorporated into bone and reduce the activity of osteoclasts.

Osteoporosis can also be treated with:

- **Denosumab:** inhibitor of RANKL (RANKL is a TNF family of cytokine that activates osteoclasts; thus, denosumab inhibits osteoclasts.)
- **Teriparatide:** synthetic PTH (When used intermittently, teriparatide has a stimulatory effect on osteoblastic bone formation.)
- Calcitonin
- Raloxifene: selective estrogen receptor modifier

Rickets and Osteomalacia

- Origin is the abnormal mineralization of bone and cartilage
- Rickets is before plate closure, osteomalacia is after plate closure.
- In rickets there is expansion of the epiphyseal plates and the most striking abnormalities are the bowing of the legs and protuberant abdomen.
- In osteomalacia, symptoms are more subtle.
- Most common cause in adults is a malabsorption disorder, e.g., celiac disease; a vitamin D deficiency can also cause
- Rarely caused by enzyme deficiencies when substrate availability is normal.

Thyroid Hormones 8

Learning Objectives

- ❑ Solve problems concerning overview of the thyroid gland
- ❑ Use knowledge of biosynthesis and transport of thyroid hormones
- ❑ Interpret scenarios on physiologic actions of thyroid hormones
- ❑ Answer questions about control of thyroid hormone secretion
- ❑ Answer questions about pathologic changes in thyroid hormone secretion

THE THYROID GLAND

In mammals, thyroid hormones are essential for normal growth and maturation. Therefore, thyroid hormones are major anabolic hormones.

Dietary intake of about 500 µg per day is typical, mainly in the form of iodide (I^-) or iodine (I). To maintain normal thyroid hormone secretion, 150 µg is the minimal intake necessary. I^- is the form absorbed from the small intestine.

- The functional unit of the thyroid gland is the follicle.
- The lumen is filled with thyroglobulin, which contain large numbers of thyroid hormone molecules.
- Surrounding the lumen are the follicle cells, which function to both synthesize and release thyroid hormones.

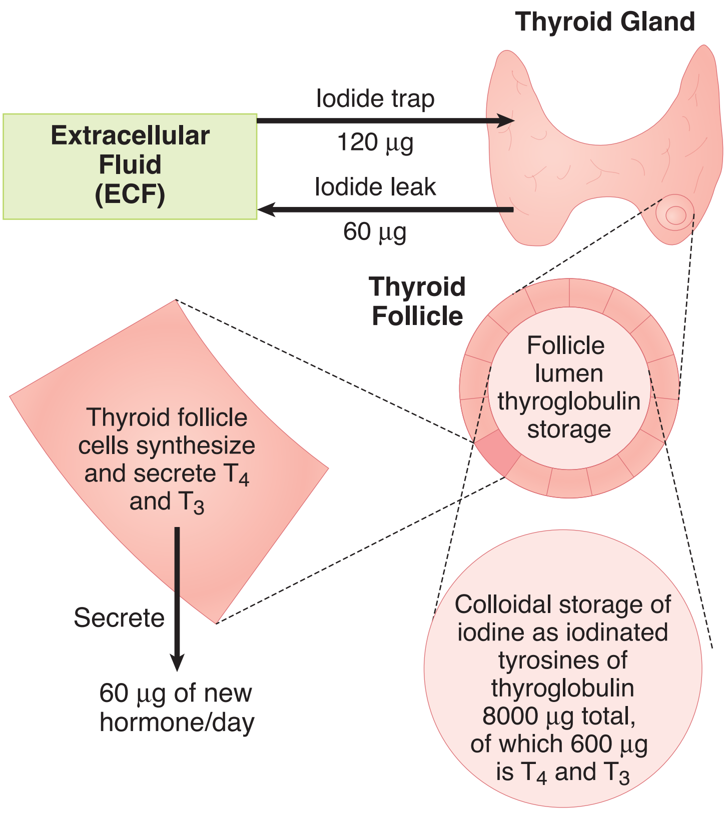


Figure VII-8-1. The Thyroid Follicle

BIOSYNTHESIS AND TRANSPORT OF THYROID HORMONE

Synthesis of Thyroid Hormone

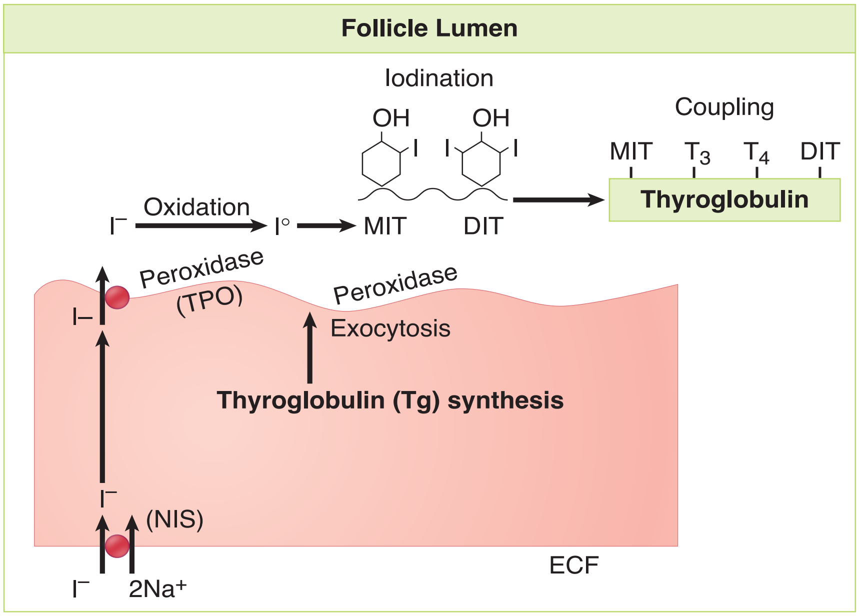


Figure VII-8-2. Steps in Thyroid Synthesis

Iodide transport

Iodine uptake is via a sodium/potassium pump powered sodium/iodide symporter on the basal membrane (NIS). This pump can raise the concentration of I^- within the cell to as much as 250x that of plasma. The pump can be blocked by anions like perchlorate and thiocyanate, which compete with I.

Along the apical membrane, the I^- is transported into the lumen by an anion exchanger called pendrin.

The 24-hour iodine uptake by the thyroid is directly proportional to thyroid function.

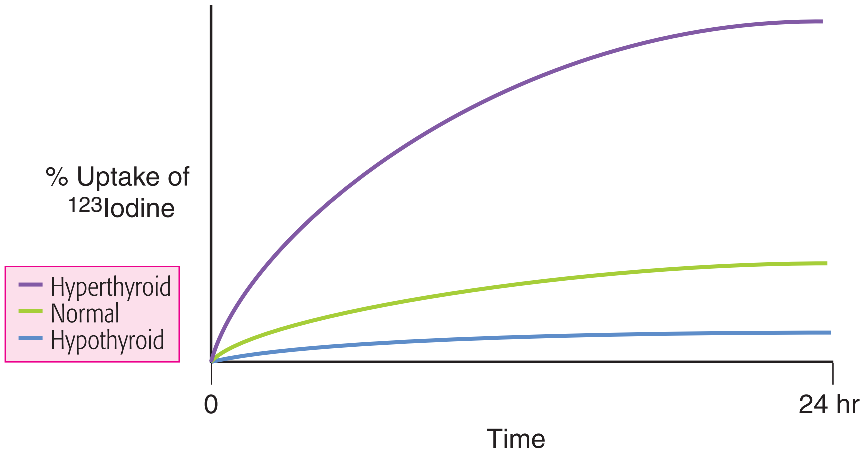


Figure VII-8-3. Relationship of Thyroid Function and Iodine Uptake

Thyroglobulin synthesis

A high molecular weight protein (>300,000 daltons) is synthesized in ribosomes, glycosylated in the endoplasmic reticulum, and packaged into vesicles in the Golgi apparatus. The thyroglobin then enters the lumen via exocytosis.

Oxidation of I^- to I^o

The enzyme thyroperoxidase (TPO), which is located at the apical border of the follicle cell, catalyzes oxidation. Peroxidase also catalyzes iodination and coupling.

Iodination

As thyroglobulin is extruded into the follicular lumen, a portion (<20%) of its tyrosine residues are iodinated. The catalyst for this reaction is peroxidase. The initial products of iodination are mono- and diiodotyrosine (MIT and DIT), respectively, with the latter form predominating, except when iodine is scarce.

Coupling

Peroxidase also promotes the coupling of iodinated tyrosine in the thyroglobulin molecule. When two DITs couple, tetraiodothyronine (T4) is formed. When one DIT and one MIT combine, triiodothyronine (T3) is formed. When iodine is abundant, mainly T4 is formed. But when iodine becomes scarce, the production of T3 increases.

Storage of thyroid hormones

Enough hormone is stored as iodinated thyroglobulin in the follicular colloid to last the body for 2–3 months.

Structure of Thyroid Hormones

The chemical structures of T4, T3, and reverse T3 (rT3) are shown below.

Note

For the exam, do not memorize structure; instead, note the number and location of iodines attached to the tyrosine residues.

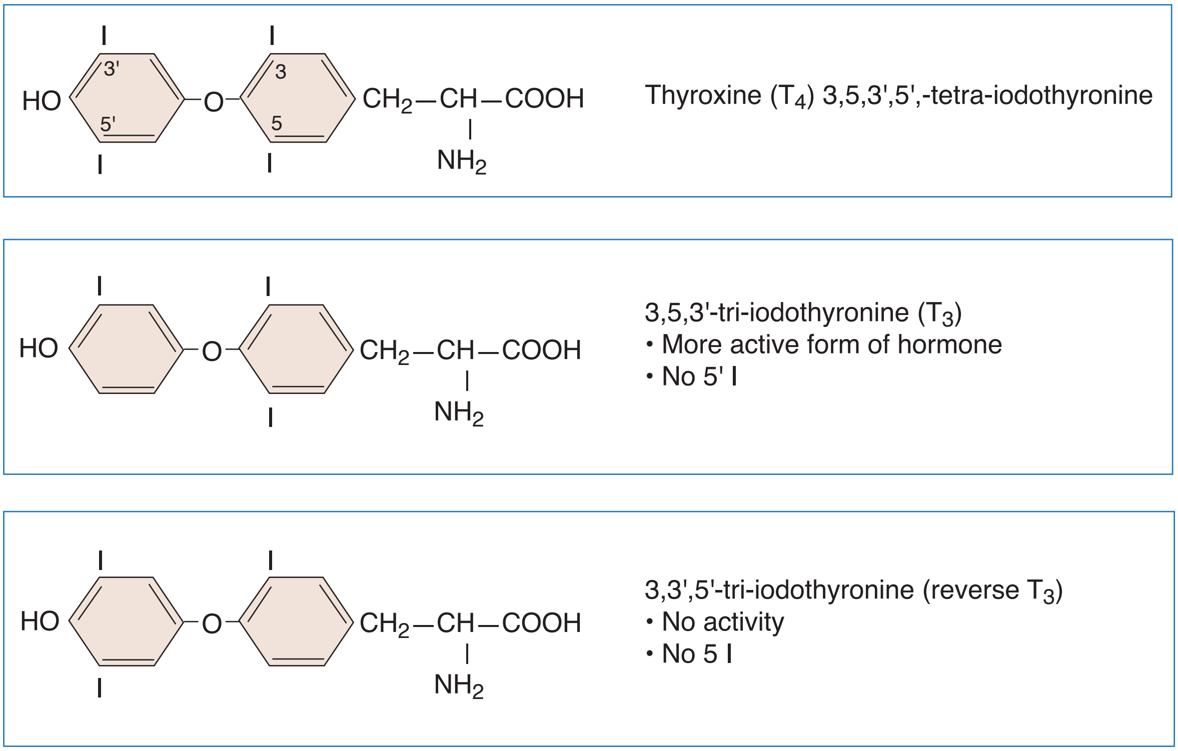


Figure VII-8-4. Active and Inactive Forms of Thyroid Hormones

Secretion of Thyroid Hormone

The figure below illustrates the main steps in thyroglobulin degradation and the release of thyroid hormones.

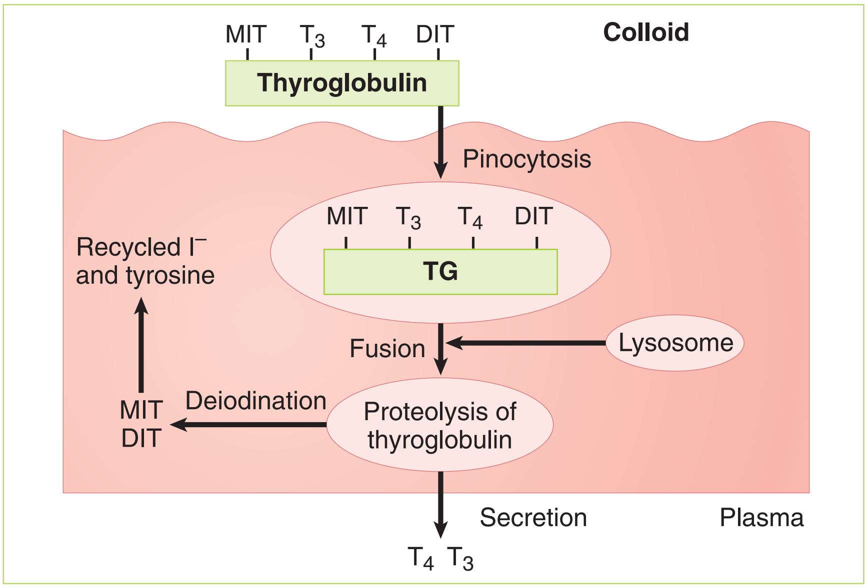


Figure VII-8-5. Secretion of Thyroid Hormone

Pinocytosis: Pieces of the follicular colloid are taken back into the follicle by endocytosis.

Fusion: The endocytosed material fuses with lysosomes, which transport it toward the basal surface of the cell.

Proteolysis of thyroglobulin: Within the lysosomes, the thyroglobulin is broken into free amino acids, some of which are T4, T3, DIT, and MIT.

Secretion: T4 and T3 are secreted into the blood, with the T4:T3 ratio being as high as 20:1. The thyroid has the same 5'-mono-deiodinase found in many peripheral tissues and in an iodine-deficient state more of the hormone can be released as T3.

Along with thyroid hormones a small amount of thyroglobulin is also released into the circulation. Its release is increased in a number of states including thyroiditis, nodular goiter, and by cancerous thyroid tissue. After the surgical removal of cancerous thyroid tissue, any residual thyroglobulin in the circulation indicates cancerous cells are still present.

Deiodination: A microsomal deiodinase removes the iodine from iodinated tyrosines (DIT and MIT) but not from the iodinated thyronines (T3 and T4). The iodine is then available for resynthesis of hormone. (Individuals with a deficiency of this enzyme are more likely to develop symptoms of iodine deficiency.)

Transport of Thyroid Hormones in Blood

There is an equilibrium between bound and free circulating thyroid hormone in the bloodstream.

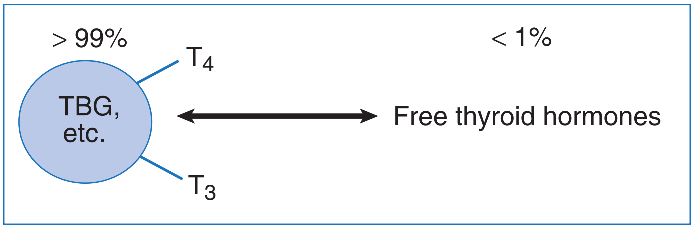


Figure VII-8-6. Plasma Transport of Thyroid Hormone

TBG = thyroid-binding globulin

About 70% of the circulating thyroid is bound to thyroid-binding globulin (TBG). The remainder of the bound protein is attached to thyroxine-binding prealbumin (transthyretin) and albumin. Large variations in TBG do not normally affect the free form. A rare congenital deficiency or excess of TBG drastically alters the bound fraction but because the free fraction is normal, the individuals are all euthyroid.

Also, T4 has the higher affinity for binding proteins; therefore, it binds more tightly to protein than does T3, and consequently has a greater half-life than T3. Most circulating thyroid hormone is T4. Normally, there is 50x more T4 than T3.

- T4 half-life = 6 days
- T3 half-life = 1 day

The amount of circulating thyroid hormone is about 3x the amount normally secreted by the thyroid gland each day. Thus, circulating protein-bound thyroid hormones act as a significant reserve.

Activation and Degradation of Thyroid Hormones

T3 and T4 bind to the same nuclear receptor, but T3 binds with 10x more affinity than T4. Because it has greater affinity for the receptor, T3 is the more active form of thyroid hormone.

- Many target tissues can regulate the conversion of T4 to either T3 or rT3, thereby locally controlling hormone activity.
- Most of the circulating T3 is derived from the peripheral conversion of T4 into T3 and its release again into the circulation (e.g., liver, kidney, and skeletal muscle).

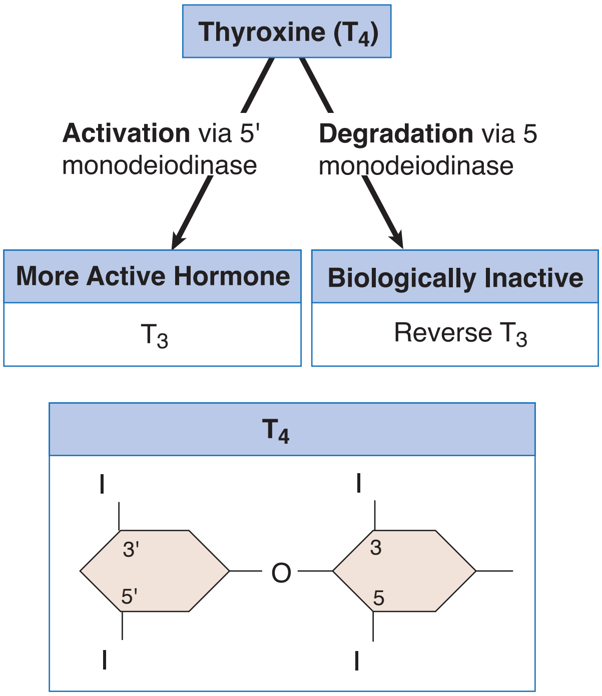


Figure VII-8-7. Peripheral Conversion of Thyroid Hormone

Certain clinical states are associated with a reduction in the conversion of T4 into T3, often with an enhanced conversion of T4 into rT3 (low T3 syndrome). Such states would include fasting, medical and surgical stresses, catabolic diseases, and even excess secretion of cortisol could be included.

The result is a reduction in metabolic rate and a conservation of energy resources. In the early stages, the circulating T4 is normal but in many cases as the metabolic problem or stress becomes more severe, T4 can fall as well.

PHYSIOLOGIC ACTIONS OF THYROID HORMONES

In many tissues, thyroid hormones are not the prime indicators or the major inhibitors of specific cellular processes. Rather, a multitude of processes function properly only when optimal amounts of thyroid hormones are present. This underscores the permissive nature of thyroid hormones.

Metabolic Rate

Thyroid hormones increase metabolic rate, as evidenced by increased O_2 consumption and heat production. Thyroid hormones increase the activity of the membrane-bound Na/K^-ATPase in many tissues, and it can be argued that it is the increased pumping of Na^+ that accounts for most of the increase in metabolic rate.

- The increase in metabolic rate produced by a single dose of T4 occurs only after a latency of several hours but may last 6 days or more.
- Thyroid hormones are absolutely necessary for normal brain maturation and essential for normal menstrual cycles. Hypothyroidism leads to menstrual irregularities (menorrhagia) and infertility (anovulatory cycles).

Growth and Maturation (T4 and T3 Anabolic Hormones)

Fetal growth rates appear normal in the absence of thyroid hormone production (i.e., if the fetus is hypothyroid). However, without adequate thyroid hormones during the perinatal period, abnormalities rapidly develop in nervous system maturation.

- Synapses develop abnormally and there is decreased dendritic branching and myelination. These abnormalities lead to mental retardation.
- These neural changes are irreversible and lead to cretinism unless replacement therapy is started soon after birth.

Lipid Metabolism

- Thyroid hormone accelerates cholesterol clearance from the plasma.
- Thyroid hormones are required for conversion of carotene to vitamin A, and, as a consequence, hypothyroid individuals can suffer from night blindness and yellowing of the skin.

CHO Metabolism

- Thyroid hormone increases the rate of glucose absorption from the small intestine.

Cardiovascular Effects

- Thyroid hormones have positive inotropic and chronotropic effects on the heart.
- The increased contractility is partly direct and partly indirect: they increase the number and affinity of β-adrenergic receptors in the heart, thereby increasing the sensitivity to catecholamines.
- Acting on the SA node, they directly increase heart rate.
- Cardiac output is increased, and both heart rate and stroke volume are elevated.
- Systolic pressure increases are due to increased stroke volume, and diastolic pressure decreases are due to decreased peripheral resistance.
- Thyroid hormones in the normal range are required for optimum cardiac performance.

Additional Effects

Thyroid hormones maintain the ventilatory response to hypoxia, increase erythropoietin, and increase gut motility and bone turnover.

Hypothyroidism is associated with an increased prolactin. TRH in excess amounts will stimulate prolactin.

CONTROL OF THYROID HORMONE SECRETION

Feedback Relationships

The overall control of thyroid function can be seen in the figure below.

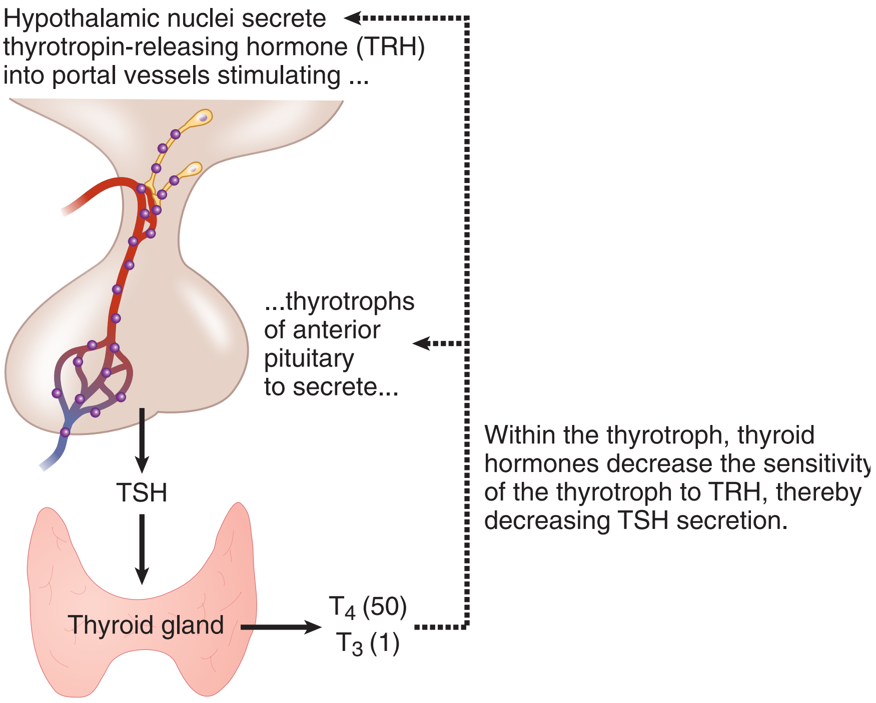


Figure VII-8-8. Hypothalamic–Pituitary Control of Thyroid-Hormone Secretion

- TRH provides a constant and necessary stimulus for TSH secretion. In the absence of TRH, the secretion of TSH (and T4) decreases to very low levels. The target tissue for TSH is the thyroid, where it increases the secretion of thyroid hormone, T4 being the predominant form.
- Negative feedback of thyroid hormones is exerted mainly at the level of the anterior pituitary gland.
- Because the main circulating form is T4, it is T4 that is responsible for most of the negative feedback.
- However, within the thyrotrophs the T4 is converted to T3 before it acts to reduce the sensitivity of the thyrotroph to TRH.
- As long as circulating free T4 remains normal, changes in circulating T3 have minimal effects on TSH secretion. However, TSH secretion increases if there is a significant drop in circulating free T4, even in the presence of an increase in circulating T3.

Overall Effects of Thyrotropin (TSH) on the Thyroid

Rapidly induced TSH effects

TSH tends to rapidly increase (within minutes or an hour) all steps in the synthesis and degradation of thyroid hormones, including:

- Iodide trapping
- Thyroglobulin synthesis and exocytosis into the follicular lumen
- Pinocytotic reuptake of iodinated thyroglobulin back into the thyroid follicular cell
- Secretion of T4 into the blood

Slowly induced TSH effects

Changes that occur more slowly (hours or days) in response to TSH include:

- Increased blood flow to the thyroid gland
- Increased hypertrophy or hyperplasia of the thyroid cells, which initially leads to increased size of the gland or goiter

Tests of Thyroid Function

- Determining the serum TSH is the first step in evaluating thyroid function.
- Secondly, free T4 (FT4) measurements are now readily available and would confirm an initial conclusion based on the TSH measurement. An alternative test would be an index of the free T4 via resin uptake.
- Autoimmune thyroid disease is sometimes detected by measuring circulating antibodies. Most notably are the TPO antibodies, which are elevated in Hashimoto's thyroiditis (hypothyroidism) and Graves' disease (hyperthyroidism).
- Additional antibodies are those against thyroglobulin and the TSI antibodies that stimulate the TSH receptor in Graves' disease.
- Uptake of iodine isotopes by the thyroid allows thyroid imaging and quantitation of tracer uptake.
- Subacute thyroiditis: overall a below-normal uptake of isotope
- Graves' disease: increased tracer uptake that is distributed evenly throughout the enlarged gland
- Toxic adenomas: local areas of increased uptake with below-normal uptake in the remainder of the gland
- Toxic multinodular goiter: enlarged gland that often has an abnormal architecture and with multiple areas of high and low uptake.

Recall Question

Which of the following is correct regarding the effects of thyroid hormone on the cardiovascular system?

A. Thyroid hormone acts to increase cardiac output by increasing chronotropic and ionotropic effects on the heart.

B. Thyroid hormone acts on the AV node to directly increase the heart rate.

C. In cases of thyroid hormone excess, total peripheral resistance increases.

D. Thyroid hormone increases the affinity of alpha adrenergic receptors in the heart.

E. In thyroid hormone excess, systolic pressure decreases while diastolic pressure increases.

Answer: A

PATHOLOGIC CHANGES IN THYROID HORMONE SECRETION

Table VII-8-1. Changes in Feedback Relationships in Several Disorders

	T_4	TSH	TRH
Primary hypothyroidism	↓	↑	↑
Pituitary hypothyroidism (secondary)	↓	↓	↑
Pituitary hyperthyroidism (secondary)	↑	↑	↓
Graves' disease (autoimmune)	↑	↓	↓

A goiter can develop in all of the disorders shown in the preceding table except secondary and tertiary hypothyroidism.

Thyroidal Response to Low Intake of Iodine

In most cases, if iodine is deficient in the diet but not absent, the individual will remain euthyroid but will develop a goiter. The adaptive sequence occurs when dietary intake of iodine is deficient. In the figure below, the sequence of events begins with 1 (decreased secretion of T4) and proceeds through 4, the development of a goiter.

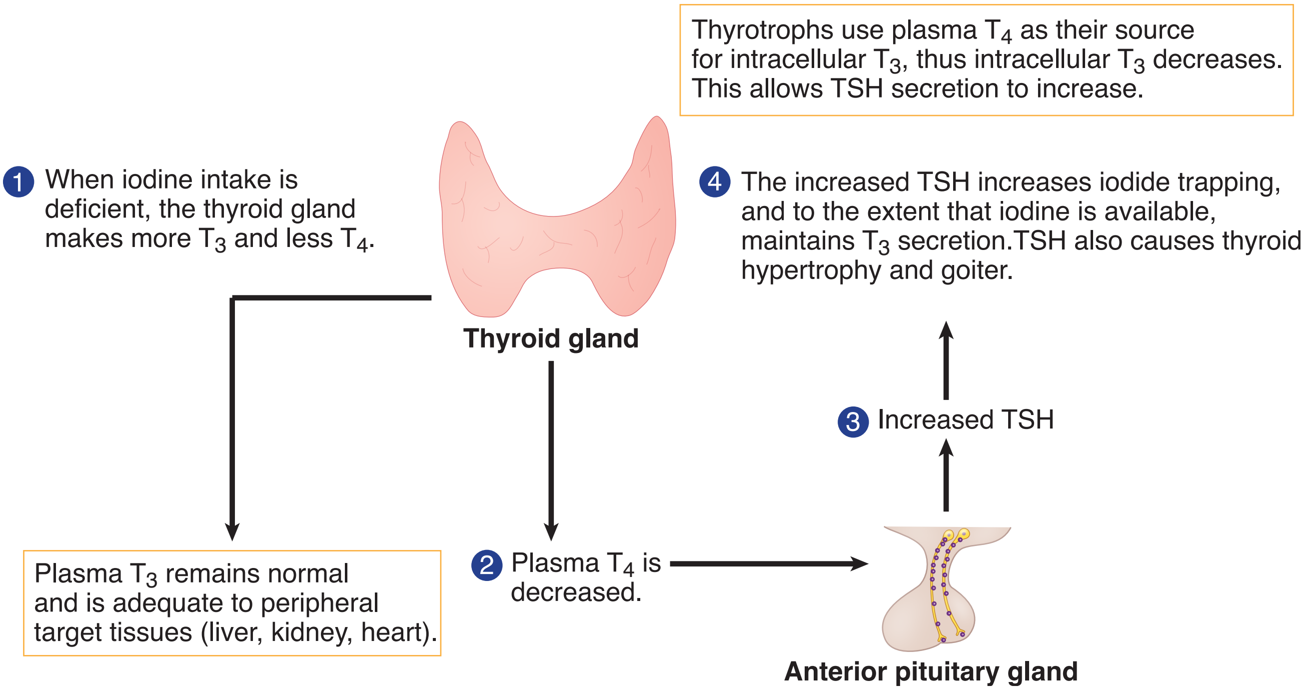


Figure VII-8-9. Iodine Deficiency

Primary Hypothyroidism

Primary changes and clinical presentation

- Most common cause is Hashimoto's thyroiditis, an autoimmune destruction of the thyroid with lymphocytic infiltration; ↑ TPO antibodies; early stages have a diffusely enlarged thyroid progressing in the later stages to a smaller atrophic and fibrotic gland.
- ↑ TSH, ↓ FT4; in subclinical hypothyroidism the TSH is on the high side of normal and the FT4 is on the low side of normal.
- Decreased basal metabolic rate and oxygen consumption
- Plasma cholesterol and other blood lipids tend to be elevated.
- Increased TRH drives a hyperprolactinemia. In women it may result in amenorrhea with galactorrhea; more often anovulatory cycles with menorrhagia. In men infertility and gynecomastia.
- Decreased GFR and an inability to excrete a water load, which may lead to hyponatremia.
- Inability to convert carotene to vitamin A may cause yellowing of the skin and night blindness.
- Slow thinking and lethargy; some patients have severe mental symptoms, dementia, or psychosis ("myxedema madness")
- Decreased food intake but individuals tend to be overweight
- Deep tendon reflexes with slow relaxation phase
- In the early stages, a decreased cardiac performance due to diminished contractility. In the later stages, cardiac features suggestive of cardiomyopathy
- Anemia, constipation, hoarseness in speech, and the skin is dry and cool
- A decreased ventilatory drive to hypercapnia and hypoxia
- Accumulation of subcutaneous mucopolysaccharides that give rise to a nonpitting edema (myxedema)
- Myxedema coma is the end stage of untreated hypothyroidism. The major features are hypoventilation, fluid and electrolyte imbalances, and hypothermia and ultimately shock and death.

Cretinism

- Untreated postnatal hypothyroidism results in cretinism, a form of dwarfism with mental retardation.
- Individuals often appear normal following delivery but may display some respiratory difficulty, jaundice, feeding problems, and hypotonia.
- Abnormalities rapidly develop in nervous system maturation, which are irreversible and result in mental retardation.
- Prepubertal growth, including bone ossification, is retarded in the absence of thyroid hormones. A stippled epiphysis is a sign of hypothyroidism in children.

- There is no evidence that thyroid hormones act directly on growth or bone formation. Rather, thyroid hormone appears to be permissive or act synergistically with growth hormone or growth factors acting directly on bone. Thyroid hormone is required for normal synthesis and secretion of growth hormone.
- Acquired hypothyroidism during childhood results in dwarfism but there is no mental retardation.
- At puberty, increased androgen secretion drives an increased growth hormone secretion. This will not occur with depressed levels of thyroid hormones.

Additional causes of hypothyroidism

- Secondary generally associated with panhypopituitarism
- Secondary or tertiary characterized by ↓ FT4 and inappropriately normal TSH.
- Severe iodine deficiency (not in the United States)
- Drug induced, e.g., lithium
- Failure to escape from the Wolff-Chaikoff effect following excessive iodine intake
- Rarely there can be resistance to thyroid hormone

Treatment

- Replacement doses of T4. The goal is to give enough T4 to normalize serum TSH.
- Because metabolism of T4 decreases and the plasma half-life increases with age, higher doses of T4 are required in younger individuals.
- Overall levels of TSH must be checked on occasion to make sure of the proper dosage of T4.
- In women beyond menopause, overprescribing T4 can contribute to the development of osteoporosis.

Primary Hyperthyroidism (Graves' Disease)

Thyrotoxicosis by definition is the clinical syndrome whereby tissues are exposed to high levels of thyroid hormone (= hyperthyroidism). The most common cause of thyrotoxicosis is Graves' disease, a primary hyperthyroidism. Graves' is an autoimmune problem in which one antibody is directed against the thyroid receptor. It is referred to as the thyroid stimulating antibody (TSI or TSH-R). TPO antibodies and those against thyroglobulin are also found in Graves'.

- Increased FT4, decreased TSH (it is the TSI stimulating the TSH receptor on the thyroid that is driving the hyperthyroidism)
- Symmetrically enlarged thyroid
- Increased radioiodine uptake by the thyroid and decreased serum cholesterol

- Only Graves' disease has thyroid-stimulating antibodies. The only types of hyperthyroidism with increased radioactive iodine uptake are Graves' disease and toxic nodular goiter.
  - Subacute thyroiditis and "silent" or "painless" thyroiditis do not have increased radioactive iodine uptake; they are "leaking" thyroid hormone out of a gland damaged by antibodies.
- Increased metabolic rate and heat production (patients tend to seek a cool environment)
- Increased cardiac output, contractility, and heart rate with possible palpitations and arrhythmias (increased β-adrenergic stimulation)
- Many symptoms suggest a state of excess catecholamines but circulating catecholamines are usually normal.
- Weight loss with increased food intake, protein wasting, and muscle weakness
- Tremor, nervousness, and excessive sweating
- Wide-eyed stare (exophthalmos) seen in patients, caused by an infiltration of orbital soft tissues and extraocular muscles and the resulting edema (this process is caused by the antibodies)

Untreated hyperthyroidism may decompensate into a condition called "thyroid storm."

The end-stage of Graves' disease is often a hypothyroidism.

Acute treatment

- Beta blockers are most rapid in effect
- Methimazole or propylthiouracil stops the production of hormone
- Iodine in high dose stops incorporation of iodine into the gland
- Steroids such as dexamethasone stop conversion of T4 to T3
- Long-term permanent cure is ablation of the gland with radioactive iodine

Additional origins of hyperthyroidism (thyrotoxicosis)

- Autonomously functioning thyroid adenoma
- Toxic multinodular goiter
- Subacute and silent thyroiditis
- TSH-secreting pituitary adenoma (secondary hyperthyroidism) (very rare)

Goiter

A goiter is simply an enlarged thyroid and does not designate functional status. It can be present in hypo-, hyper-, and euthyroid states.

There is no correlation between thyroid size and function.

- A generalized enlargement of the thyroid is considered a "diffuse goiter."
- Diffuse enlargement often results from prolonged stimulation by TSH or TSH-like factor; e.g., Hashimoto's thyroiditis, Graves' disease, diet deficient in iodine
- An irregular or lumpy enlargement of the thyroid is considered a "nodular goiter."
- With time, excessive stimulation by TSH can result in a multinodular goiter e.g. iodine deficiency initially produces a diffuse nontoxic goiter. Long term however, focal hyperplasia with necrosis and hemorrhage results in the formation of nodules. Nodules vary from "hot nodules" that can trap iodine to "cold nodules" that cannot trap iodine.

Growth, Growth Hormone, and Puberty

9

Learning Objectives

- ❑ Explain information related to in-utero and prepubertal growth
- ❑ Explain information related to physiologic actions of growth hormone
- ❑ Use knowledge of control of growth hormone secretion
- ❑ Answer questions about puberty
- ❑ Use knowledge of acromegaly

IN-UTERO AND PREPUBERTAL GROWTH

Intrauterine Growth

- Important roles for growth hormone, IGF-II (early in gestation), IGF-I (later in gestation) and insulin
- Infants of diabetic mothers have increased insulin levels and are large.
- Smoking decreases vascularity of the placenta and decreases birth weight.
- Poor maternal nutrition leading cause of low birth weight worldwide.

Postnatal Growth

- Although fetal hypothyroidism does not decrease birth weight, hypothyroidism following delivery causes irreversible abnormalities in nervous system maturation, which in turn lead to mental retardation (cretinism).
- Growth hormone, insulin, and thyroid hormone play major roles. Acquired hypothyroidism later in childhood will slow growth and reduce bone advancement more than growth hormone deficiency, but will not cause mental retardation.
- Replacement of hormone deficiencies creates a period of catch-up growth, but it is soon replaced with a normal growth rate.
- There is no major role for gonadal sex steroids on prepubertal growth or for glucocorticoids but glucocorticoid excess will slow growth.
- Hypersecretion of growth hormone pre-puberty (pituitary adenoma) results in giantism. It also delays pubertal changes, and the subsequent hypogonadism contributes to the giantism.

Prepubertal Growth Hormone Deficiency

- Deficiencies can be congenital (decreased birth length), idiopathic (low GHRH), or acquired (hypothalamic-pituitary tumor).
- A deficiency causes dwarfism, which is characterized by: short stature, chubby, immature facial appearance, delayed skeletal maturation, and tendency to episodes of hypoglycemia.
- Tissue resistance to growth hormone (↑ growth hormone, ↓ IGF-I) results in Laron syndrome (Laron dwarfism).
- Stimulation test is with an arginine infusion.
- Growth hormone deficiency following puberty decreases lean body mass, and replacement therapy is now considered an acceptable treatment.
- Treatment of GH deficiency is simple replacement of GH.
- Treatment of Laron dwarfism (lack of GH receptor) is synthetic IGF. Mecasermin is the name of recombinant IGF.

PHYSIOLOGIC ACTIONS OF GROWTH HORMONE

Growth hormone is a major anabolic growth-promoting hormone and a stress hormone. All anabolic hormones (i.e., growth hormone, insulin, thyroid hormones, and androgens) are required for normal growth. The major stress and anabolic actions of growth hormone are shown below. The figure shows that most of the direct actions of growth hormone are consistent with its actions as a stress hormone.

A direct anabolic action is the promotion of amino acid entry into cells, thus making them more available for protein synthesis. However, most of the anabolic actions of growth hormone are indirect via the production of growth factors.

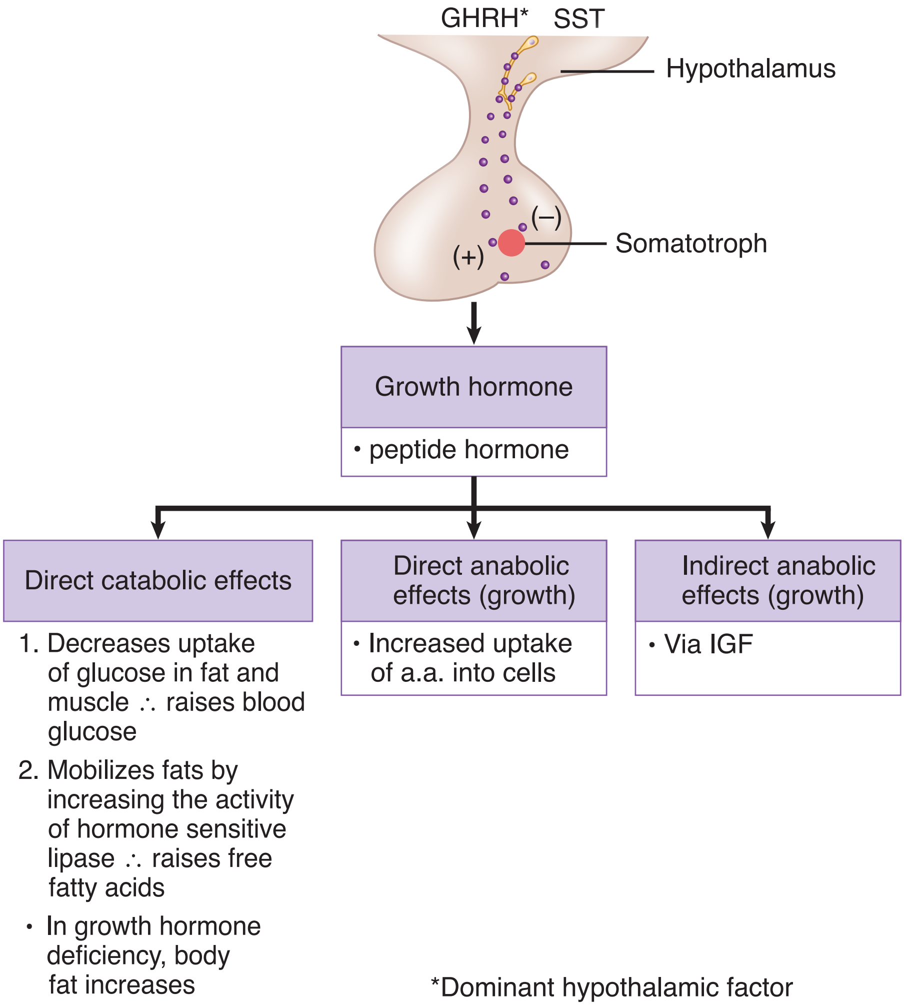


Figure VII-9-1. Overview of Growth Hormone

GHRH = growth hormone-releasing hormone
SST = somatostatin

Indirect Anabolic Actions of Growth Hormone

Most of the anabolic actions of growth hormone are an indirect result of increased production of insulin-like growth factors (IGFs). A major growth factor is IGF-I.

The steps in the production and release of IGF-I are shown below.

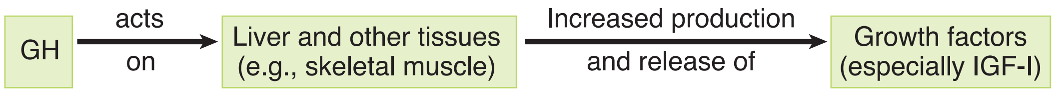


Figure VII-9-2. IGF-Mediated Effects of Growth Hormone

Specific Properties of the IGFs

IGF-I is a major anabolic growth factor. It has the following characteristics:

- A circulating peptide growth factor similar in structure to proinsulin and has some insulin-like activity.
- Circulates in the blood tightly bound to a large protein, whose production is also dependent on growth hormone. Protein binding increases the half-life and thus serves as a better 24-hour marker of GH (half-life 15–20 minutes).
- The major known anabolic effect of IGF-I is that it increases the synthesis of cartilage (chondrogenesis) in the epiphyseal plates of long bones, thereby increasing bone length.
- It is also hypothesized that circulating IGFs increase lean body mass. The decreased lean body mass of aging may, in part, be due to the concomitant decrease in IGFs. IGFs also decrease in catabolic states, especially protein-calorie malnutrition.
- IGF-II is another growth factor, the importance of which is not well understood but may have a role in fetal development.

CONTROL OF GROWTH HORMONE (GH) SECRETION

- GH secretion is pulsatile. The secretory pulses are much more likely to occur during the night in stages III and IV (non-REM) of sleep than during the day.
- Secretion of GH requires the presence of normal plasma levels of thyroid hormones. GH secretion is markedly reduced in hypothyroid individuals.
- During the sixth decade of life and later, GH secretion diminishes considerably in both men and women. What initiates this decrease is unknown.

Each of the promoters could act by increasing GHRH secretion, decreasing SST secretion, or both.

Notice that most of the factors that regulate GH secretion are identical to those that regulate glucagon (except for those boxed). These factors are consistent with their shared role as stress hormones.

The inhibitory effect of IGF-I represents a negative feedback loop to the hypothalamus.

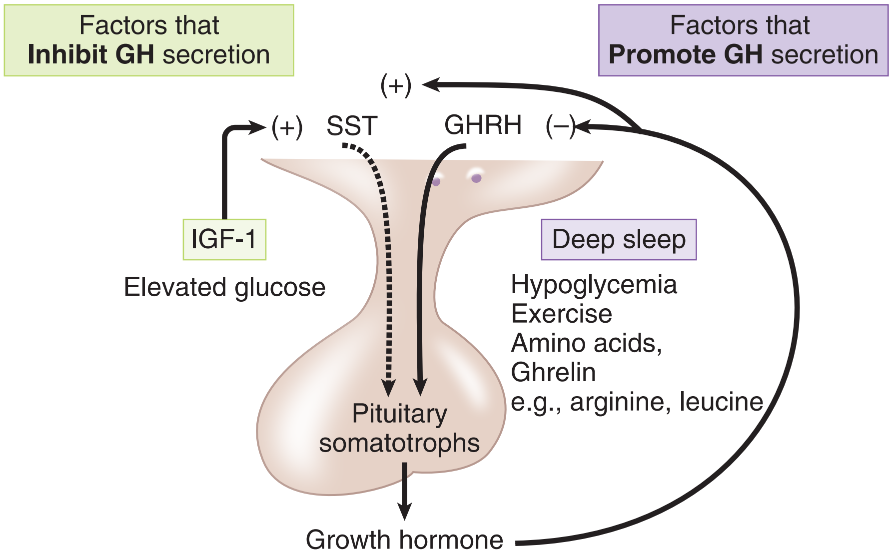


Figure VII-9-3. Control of Growth Hormone Secretion

SST: somatostatin

PUBERTY

Reproductive Changes

Hypothalamic pulse generator increases activity just before physical changes at puberty.

First noted sign in a female is breast development; first by estrogen (promotes duct growth) then progesterone (promotes development of milk-producing alveolar cells). First noted sign in a male is enlargement of the testes (mainly FSH stimulating seminiferous tubules).

Pubic hair development in males and females is dependent on androgen.

Growth Changes

During puberty, androgens promote the secretion in the following anabolic sequence: **At puberty, if T4 is normal, ↑ androgens drive ↑ growth hormone, which drives ↑ IGF-I**.

- IGF-I is the major stimulus for cell division of the cartilage-synthesizing cells located in the epiphyseal plates of long bones.
- In males, the increased androgen arises from the testes (testosterone); in females, from the adrenals (adrenarche).
- Near the end of puberty, androgens promote the mineralization (fusion or closure) of the epiphyseal plates of long bones. Estrogen can also cause plate closure, even in men.
- In females, the growth spurt begins early in puberty and is near completion by menarche. In males, the growth spurt develops near the end of puberty.

ACROMEGALY

Acromegaly is caused by a post-pubertal excessive secretion of growth hormone. It is almost always due to a macroadenoma (>1 cm diameter) of the anterior pituitary and second in frequency to prolactinomas.

- Slow onset of symptoms; disease usually present for 5–10 years before diagnosis
- Ectopic GHRH secretion (rare)
- Some tumors contain lactotrophs, and elevated prolactin can cause hypogonadism and galactorrhea.
- Increased IGF-I causes most of the deleterious effects of acromegaly but growth hormone excess directly causes the hyperglycemia and insulin resistance.
- Characteristic proliferation of cartilage, bone and soft tissue, visceral, and cardiomegaly
- Observable changes include enlargement of the hands and feet (acral parts) and coarsening of the facial features, including downward and forward growth of the mandible. Also, increased hat size.
- Measurement of IGF-I is a useful screening measure and confirms diagnosis with the lack of growth hormone suppression by oral glucose.
- Diagnosis: confirm the following before treatment is started: **elevated IGF, failed suppression of GH/IGF after giving glucose, MRI showing lesion in brain in pituitary**
- Never start with a scan in endocrinology. Benign pituitary "incidentaloma" is common in 2–10% of the population. Always confirm the presence of an overproduction of a hormone before doing a scan. This is true for adrenal lesions as well.
- Treatment:
  - Surgical removal by trans-sphenoidal approach is first. Removal of an over-producing adenoma is the first treatment in most of endocrinology with the exception of prolactinoma.
  - If surgical removal fails, use the growth hormone receptor antagonist, pegvisomant, or octreotide. Octreotide is synthetic somatostatin. Cabergoline is a dopamine agonist used when other medications have failed.
  - Radiation is used last, only after surgery, pegvisomant, octreotide and cabergoline have failed.

Recall Question

Which of the following is correct about the control of growth hormone (GH) secretion?

A. Continuous and slow

B. Occurs in the early stages of sleep during stage 1 and 2

C. Depends on thyroid hormone plasma levels

D. Accelerates during decade 6 of life

E. Depends on plasma insulin levels

Answer: C

Male Reproductive System 10

Learning Objectives

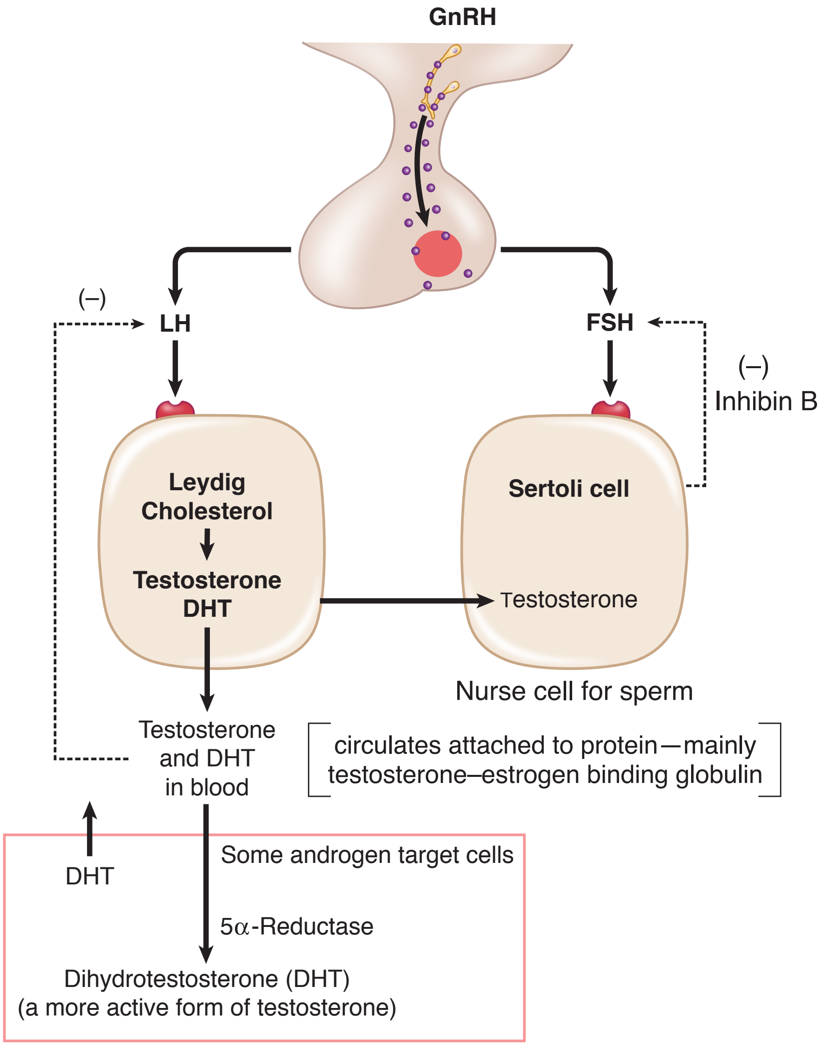
- ❑ Solve problems concerning hypothalamic-pituitary-gonadal axis in males
- ❑ Solve problems concerning age-related hormonal changes in males
- ❑ Demonstrate understanding of erection, emission, and ejaculation
- ❑ Use knowledge of gonadal dysfunction in the male

HYPOTHALAMIC-PITUITARY-GONADAL (HPG) AXIS IN MALES

The factors involved in the overall control of adult male hormone secretion can be seen below.

Note

LH, FSH, TSH, and human chorionic gonadotropin (hCG) are glycoproteins with identical alpha subunits. The beta subunits differ and thus confer specificity.



GnRH—synthesized in preoptic region of hypothalamus and secreted in pulses into hypophyseal portal vessels • produces pulsatile release of LH and FSH • pulsatile release of GnRH prevents downregulation of its receptors in anterior pituitary
LH and **FSH**—produced and secreted by gonadotrophs of anterior pituitary • LH stimulates Leydig cells to produce testosterone. • FSH stimulates Sertoli cells (see below).
Leydig cell testosterone—some diffuses directly to Sertoli cells, where it is required for Sertoli cell function. • produces negative feedback for LH
Sertoli cell inhibin B—produces negative feedback for FSH

Figure VII-10-1. Control of Testes

LH/Leydig Cells

Leydig cells express receptors for luteinizing hormone (LH). LH is a peptide hormone that activates Gs--cAMP, which in turn initiates testosterone production by activating steroidogenic acute regulatory protein (StAR).

- Testosterone diffuses into Sertoli cells (high concentration) and into the blood.
- Circulating testosterone provides negative feedback to regulate LH secretion at the level of the hypothalamus and anterior pituitary.
- Leydig cells aromatize some of this testosterone into estradiol.

5α-reductase

Some target tissue express the enzyme 5α-reductase, which converts testosterone into the more potent dihydrotestosterone. Some important physiologic effects primarily mediated by dihydrotestosterone are as follows:

- Sexual differentiation: differentiation to form male external genitalia
- Growth of the prostate
- Male-pattern baldness
- Increased activity of sebaceous glands
- Synthesis of NO synthase in penile tissue

FSH/Sertoli Cells

FSH binds to Sertoli cells and activates a Gs--cAMP pathway. Sertoli cells release inhibin B, which has negative feedback on FSH secretion.

Note

Sertoli cells provide the nourishment required for normal spermatogenesis.

- FSH, along with a very high level of testosterone from the neighboring Leydig cells, produces growth factors necessary for growth and maturation of the sperm.
- FSH and testosterone induce the synthesis of androgen binding protein, which helps maintain high local levels of testosterone.
- Leydig cells express aromatase, which aromatizes testosterone into estradiol, an important hormone for growth and maturation of the sperm.
- Sertoli cells secrete inhibin B, which produces feedback regulation on FSH.

Hormonal Control of Testicular Function

The figure below illustrates the source and nature of the hormones controlling testicular function.

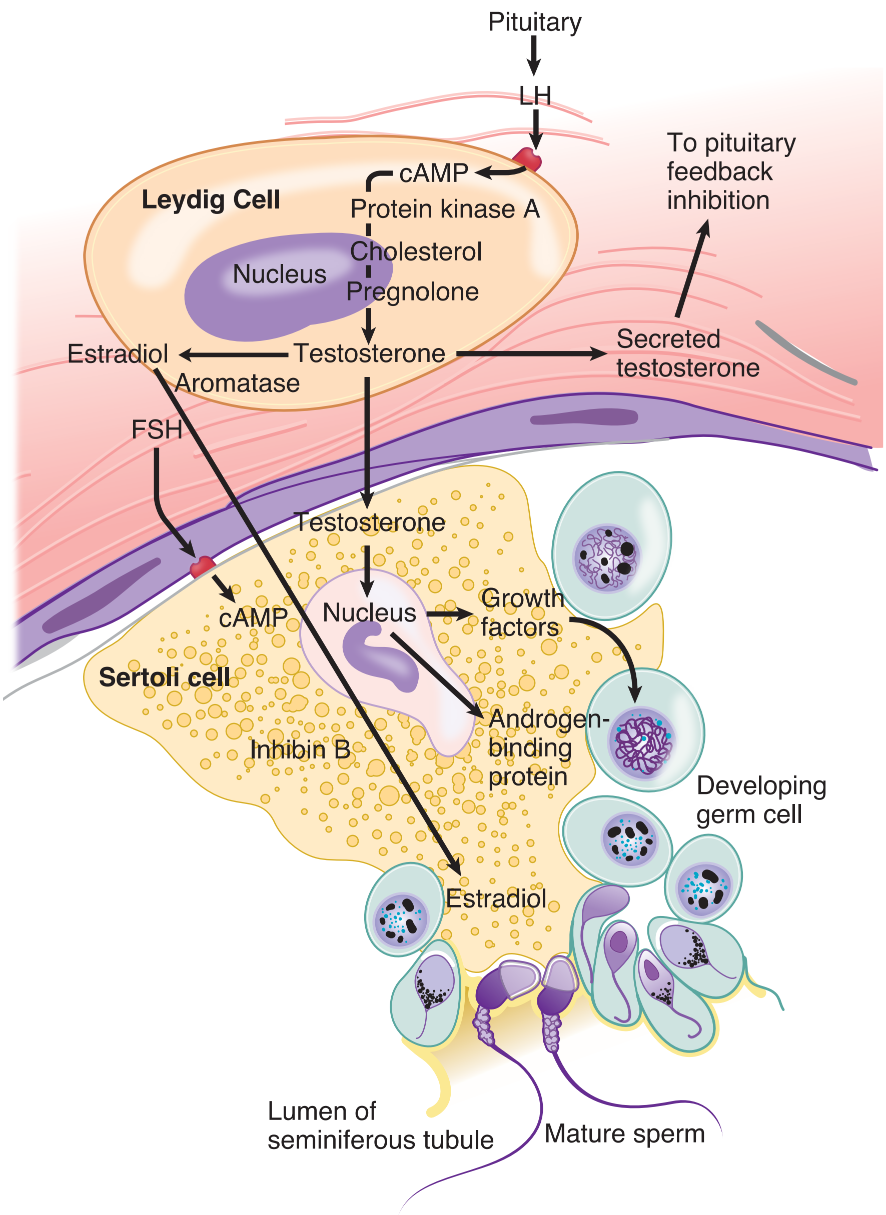


Figure VII-10-2. Endocrine Function of Testes

Definitions

Androgen: any steroid that controls the development and maintenance of masculine characteristics

Testosterone: a natural male androgen of testicular origin, controlled by the LH

Dihydrotestosterone: a more active form of testosterone made by 5-alpha-reductase. Dihydrotestosterone makes the penis, prostate, and scrotum on an embryo.

Methyl testosterone: a synthetic androgen, which is an anabolic steroid sometimes used by athletes

Adrenal androgens: natural weak androgens (male and female) of adrenal origin, controlled by ACTH. These are DHEA and androstenedione.

Inhibins: peptide hormones secreted into the blood. They inhibit the secretion of FSH by pituitary gonadotrophs.

Aromatase: an enzyme that stimulates the aromatization of the A-ring of testosterone, converting it into estradiol. The physiologic importance of this conversion is not understood; however, approximately a third of the estradiol in the blood of men arises from Sertoli cells, and the remainder arises from peripheral conversion of testosterone to estradiol by an aromatase present in adipose tissue. One sign of a Sertoli cell tumor is excessive estradiol in the blood of the affected man.

AGE-RELATED HORMONAL CHANGES IN MALES

The relative plasma LH and testosterone concentrations throughout the life of the normal human male can be seen below.

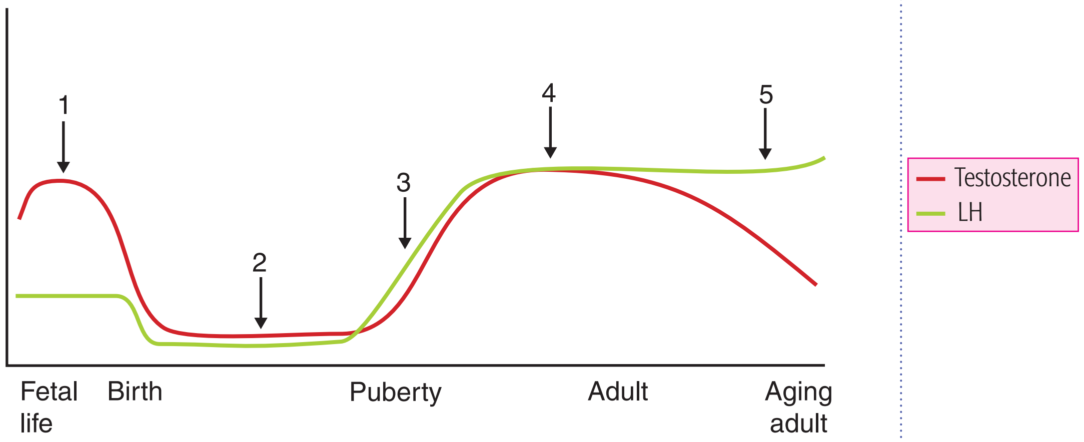


Figure VII-10-3. Development and Aging in Male Reproduction

1: Fetal life

The development of male and female internal and external structures depends on the fetal hormonal environment. The Wolffian and Müllerian ducts are initially present in both male and female fetuses. If there is no hormonal input (the situation in the normal female fetus), female internal and female external structures develop (Müllerian ducts develop, Wolffian ducts regress).

Normal male development requires the presence of 3 hormones: testosterone, dihydrotestosterone, and the Müllerian inhibiting factor (MIF).

- (hCG) + LH → Leydig cells → testosterone → Wolffian ducts
- testosterone $\xrightarrow{\text{5-}\alpha\text{-reductase}}$ dihydrotestosterone → urogenital sinus & genital organs
- Sertoli cells → MIF → absence of female internal structures

MIF prevents the development of the Müllerian ducts, which would otherwise differentiate into female internal structures. In the absence of MIF, the Müllerian ducts develop. Thus, in addition to normal male structures, a uterus will be present.

- Wolffian ducts differentiate into the majority of male internal structures; namely, epididymis, vas deferens, and seminal vesicles.
  - In the absence of testosterone, the Wolffian ducts regress.
- Dihydrotestosterone induces the urogenital sinus and genital tubercle to differentiate into the external scrotum, penis, and prostate gland.
  - In the absence of dihydrotestosterone, female external structures develop.

2: Childhood

Within a few months after birth, LH and testosterone drop to low levels and remain low until puberty. The cause of this prolonged quiescence of reproductive hormone secretion during childhood is not known. Interestingly, LH secretion remains low in spite of low testosterone.

3: Puberty

Near the onset of puberty, the amplitude of the LH pulses becomes greater, driving the mean level of LH higher. Early in puberty, this potentiation of the LH pulses is especially pronounced during sleep. This increased LH stimulates the Leydig cells to again secrete testosterone.

4: Adult

During adulthood, LH secretion drives testosterone secretion. Thus, it is not surprising that the relative levels of the two hormones parallel one another.

5: Aging adult

Testosterone and inhibin secretions decrease with age. Men in their seventies generally secrete only 60–70% as much testosterone as do men in their twenties. Nevertheless, there is no abrupt decrease in testosterone secretion in men that parallels the relatively abrupt decrease in estrogen secretion that women experience at menopause. The loss of feedback will cause an increase in LH and FSH secretion.

Effect on Muscle Mass

The capacity of androgens to stimulate protein synthesis and decrease protein breakdown, especially in muscle, is responsible for the larger muscle mass in men as compared with women. Exogenous androgens (anabolic steroids) are sometimes taken by men and women in an attempt to increase muscle mass.

Spermatogenesis Is Temperature Dependent

Effect on fertility

For unknown reasons, spermatogenesis ceases at temperatures typical of the abdominal cavity. Thus, when the testes fail to descend before or shortly after birth, and the condition (cryptorchidism) is not surgically corrected, infertility results.

Cooling mechanisms

Normally, the scrotum provides an environment that is 4°C cooler than the abdominal cavity. The cooling is accomplished by a countercurrent heat exchanger located in the spermatic cord. Also, the temperature of the scrotum and the testes is regulated by relative degree of contraction or relaxation of the cremasteric muscles and scrotal skin rugae that surround and suspend the testes.

Effect on FSH and LH

Sertoli cells, and therefore germ cell maturation, are adversely affected by the elevated temperatures of cryptorchid testes. In adults with bilaterally undescended testes, FSH secretion is elevated, probably as a result of decreased Sertoli cell production of inhibins. Testosterone secretion by the Leydig cells of cryptorchid testes also tends to be low, and as a result, LH secretion of adults with bilateral cryptorchidism is elevated.

ERECTION, EMISSION, AND EJACULATION

Erection

Erection is caused by dilation of the blood vessels (a parasympathetic response) in the erectile tissue of the penis (the corpora- and ischiocavernous sinuses). This dilation increases the inflow of blood so much that the penile veins get compressed between the engorged cavernous spaces and the Buck's and dartos fasciae.

Nitric oxide (NO), working through cGMP, mediates the vasodilation.

Emission

Emission is the movement of semen from the epididymis, vas deferens, seminal vesicles, and prostate to the ejaculatory ducts. The movement is mediated by sympathetic (thoracolumbar) adrenergic transmitters.

- Simultaneously with emission, there is also a sympathetic adrenergic-mediated contraction of the internal sphincter of the bladder, which prevents retrograde ejaculation of semen into the bladder. Destruction of this sphincter by prostatectomy often results in retrograde ejaculation.
- Emission normally precedes ejaculation but also continues during ejaculation.

Ejaculation

Ejaculation is caused by the rhythmic contraction of the bulbospongiosus and the ischiocavernous muscles, which surround the base of the penis. Contraction of these striated muscles that are innervated by somatic motor nerves causes the semen to exit rapidly in the direction of least resistance, i.e., outwardly through the urethra.

GONADAL DYSFUNCTION IN THE MALE

The consequences of deficient testosterone production depend upon the age of onset:

- Testosterone deficiency in the second to third month of gestation results in varying degrees of ambiguity in the male genitalia and male pseudohermaphrodism.
- Testosterone deficiency in the third trimester leads to problems in testicular descent (cryptorchidism) along with micropenis.
- Pubertal testosterone deficiency leads to poor secondary sexual development and overall eunuchoid features.
- Postpubertal testosterone deficiency leads to decreased libido, erectile dysfunction, decrease in facial and body hair growth, low energy, and infertility.

Causes of Hypogonadism

- Noonan syndrome
- Klinefelter's syndrome
- Hypothalamic-pituitary disorders (Kallman's syndrome, panhypopituitarism)
- Gonadal failure/sex steroid synthesis failure

Definitions

- Pseudohermaphrodite: an individual with the genetic constitution and gonads of one sex and the genitalia of the other.
- Female pseudohermaphroditism: female fetus exposed to androgens during the 8th to 13th week of development, e.g., congenital virilizing adrenal hyperplasia.
- Male pseudohermaphroditism: lack of androgen activity in male fetus, e.g., defective testes, androgen resistance
- When the loss of receptor function is complete, testicular feminizing syndrome results. Here MIF is present and testosterone is secreted, usually at elevated levels. The external structures are female, but the vagina ends blindly because there are no female internal structures.

Table VII-10-1. Hormonal Changes in Specific Altered States

	Sex Steroids	LH	FSH
Primary hypogonadism	↓	↑	↑
Pituitary hypogonadism	↓	↓	↓
Kallman's (↓ GnRH)	↓	↓	↓
Postmenopausal women	↓	↑	↑
Anabolic steroid therapy (male)*	↑	↓	(↓)
Inhibin infusion (male)†	–	–	↓
GnRH infusion (constant rate)‡	↓	↓	↓
GnRH infusion (pulsatile)	↑	↑	↑

*LH suppression causes Leydig cell atrophy in an adult male and therefore reduced testicular androgen production. Because Leydig cell testosterone is required for spermatogenesis, anabolic steroids suppress spermatogenesis.

Although testosterone is not the normal feedback regulating FSH, high circulating testosterone activity will suppress the release of FSH.

†Because FSH is required for spermatogenesis, giving inhibin suppresses spermatogenesis.

‡A constant rate of infusion of the gonadotropin-releasing hormone (GnRH) will cause a transient increase in LH and FSH secretion, followed by a decrease caused by the downregulation of gonadotroph receptors.

Recall Question

Which of the following is correct about the physiologic function of aromatase?

A. It is an enzyme that stimulates the conversion of testosterone into estradiol.

B. It is a natural weak androgen.

C. It controls and maintains the masculine characteristics.

D. It is responsible for male erection.

E. A deficiency of it causes Noonan syndrome.

Answer: A

Female Reproductive System 11

Learning Objectives

- ❑ Interpret scenarios on menstrual cycle
- ❑ Explain information related to female sex steroid metabolism and excretion
- ❑ Answer questions about menstrual irregularities
- ❑ Explain information related to pregnancy
- ❑ Solve problems concerning lactation

MENSTRUAL CYCLE

The Phases

The menstrual cycle (~28 days) can be divided into the following phases or events:

- **Follicular phase** (first 2 weeks) is also called the proliferative or preovulatory phase. This phase is dominated by the peripheral effects of estrogen, which include the replacement of the endometrial cells lost during menses.
- **Ovulation** (~day 14) is preceded by the LH surge, which induces ovulation.
- **Luteal phase** (~2 weeks) is dominated by the elevated plasma levels of progesterone, and along with lower levels of secreted estrogen, creates a secretory quiescent endometrium that prepares the uterus for implantation.
- **Menses.** Withdrawal of the hormonal support of the endometrium at this time causes necrosis and menstruation.

Note

By convention, the **first day of bleeding** (menses) is called **day 1 of the menstrual cycle.**

Follicular phase (~days 1–14)

- During the follicular phase, FSH secretion is slightly elevated, causing proliferation of granulosa cells and increased estrogen secretion within a cohort of follicles.
- One follicle has greater cellular growth and secretes more estradiol (dominant follicle). Estradiol promotes growth and increased sensitivity to FSH; thus the follicle continues to develop. The remaining follicles, lacking sufficient FSH, synthesize only androgen and become atretic (die).

The graphs below illustrate the plasma hormonal levels throughout the menstrual cycle. The length of the menstrual cycle varies, but an average length is 28 days.

Each plasma hormone concentration is plotted relative to the day on which its concentration is lowest, i.e., just prior to menses (day 28).

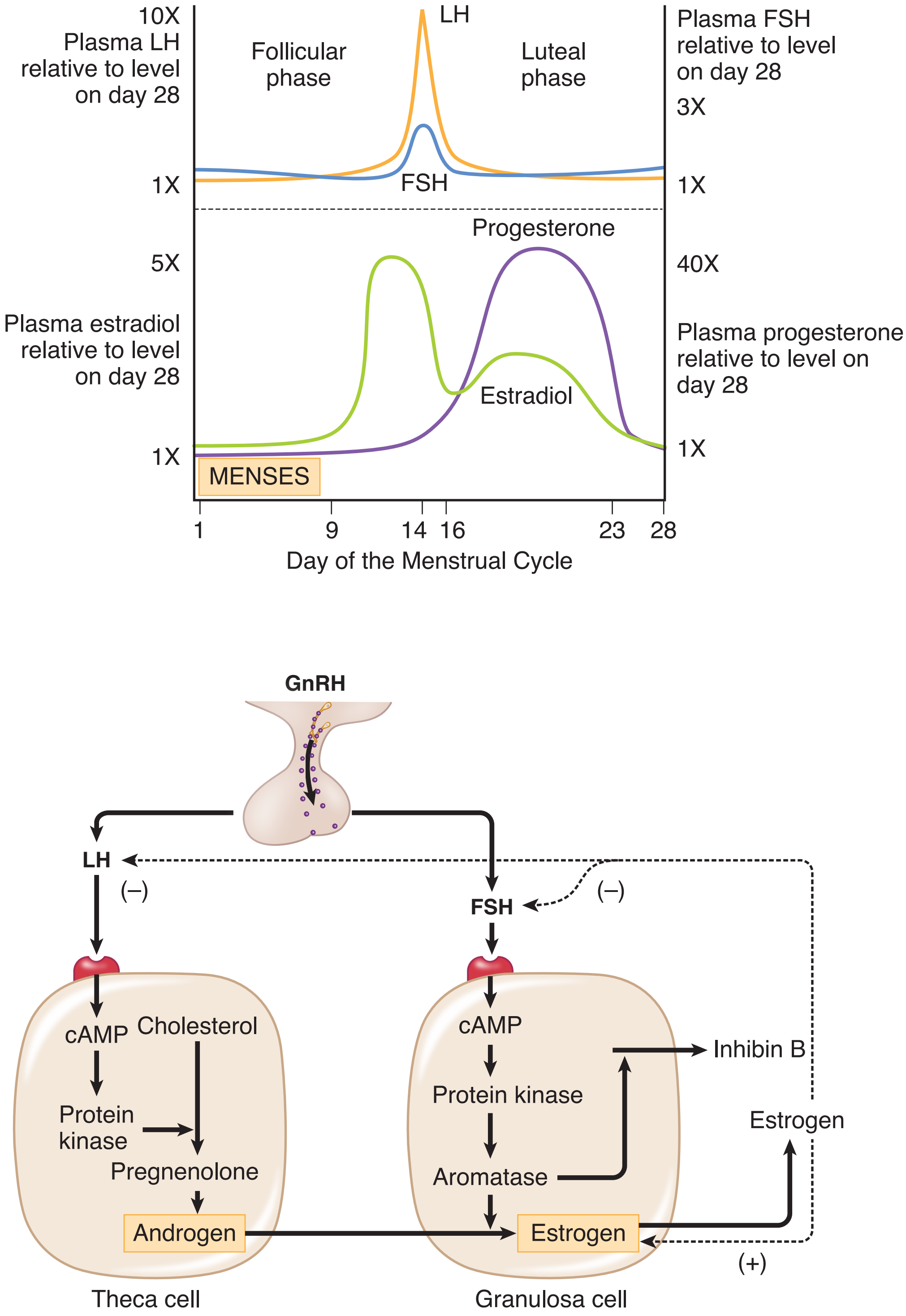


Figure VII-11-1. Follicular Phase Relationships (Approximately Days 1–14)

Theca Cells: Under LH stimulation, which acts intracellularly via cAMP, cholesterol is transported into the mitochondria (StAR is activated). The pathway continues through intermediates to androgens. Little androgen is secreted into the blood; most of the androgen enters the adjacent granulosa cells.

Granulosa Cells: Possess the follicle's only FSH receptors. When coupled to FSH, these act via cAMP to increase the activity of aromatase; aromatase converts the androgens to estrogens (mainly estradiol).

Estrogen: Some of the estrogen produced by the granulosa cells is released into the blood and inhibits the release of LH and FSH from the anterior pituitary. However, another fraction of the estrogen acts locally on granulosa cells, increasing their proliferation and sensitivity to FSH.

- This local positive effect of estrogens causes a rising level of circulating estrogens during the follicular phase, but at the same time FSH is decreasing because of the inhibitory effect of estrogen on FSH release.
- Granulosa cells also release inhibin B.
- Inhibin B inhibits the secretion of FSH by the pituitary but their role in the menstral cycle is poorly understood.

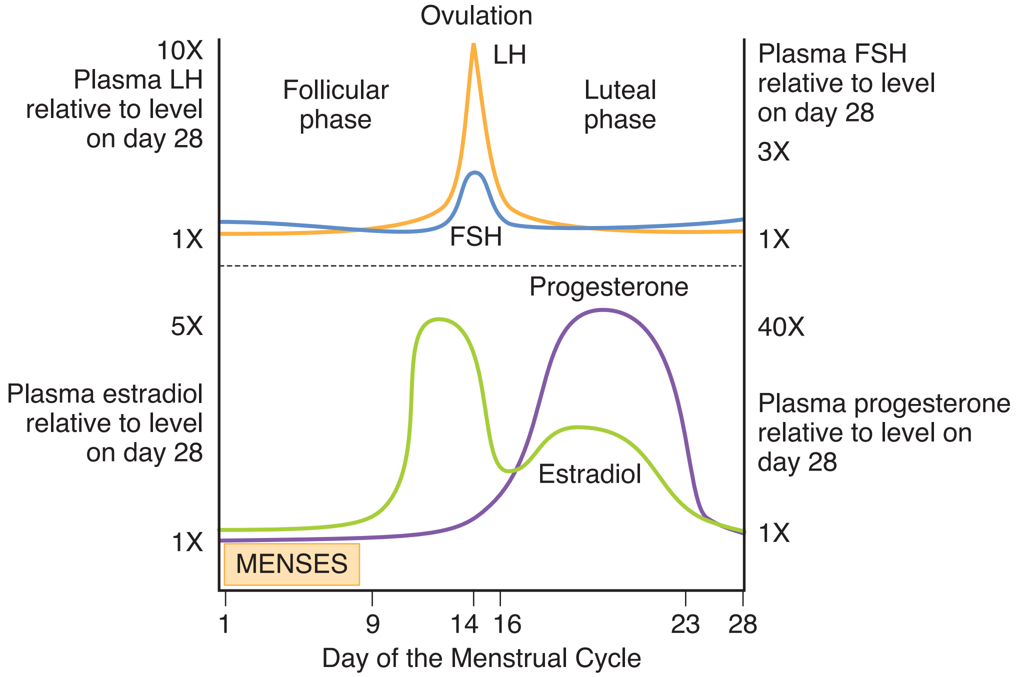
Peripheral effects of estrogen produced by the granulosa cells during the follicular phase include:

- Circulating estrogens stimulate the female sex accessory organs and secondary sex characteristics.
- Rising levels of estrogens cause the endometrial cells of the uterine mucosal layers to increase their rate of mitotic division (proliferate).
- Circulating estrogens cause the cervical mucus to be thin and watery, making the cervix easy for sperm to traverse.

Ovulation

Ovulation takes place ~day 14. This is an approximation. Since ovulation is always 14 days before the end of the cycle, you can subtract 14 from the cycle length to find the day of ovulation.

Cycle length − 14 = ovulation day



Ovulation occurs approximately day 14

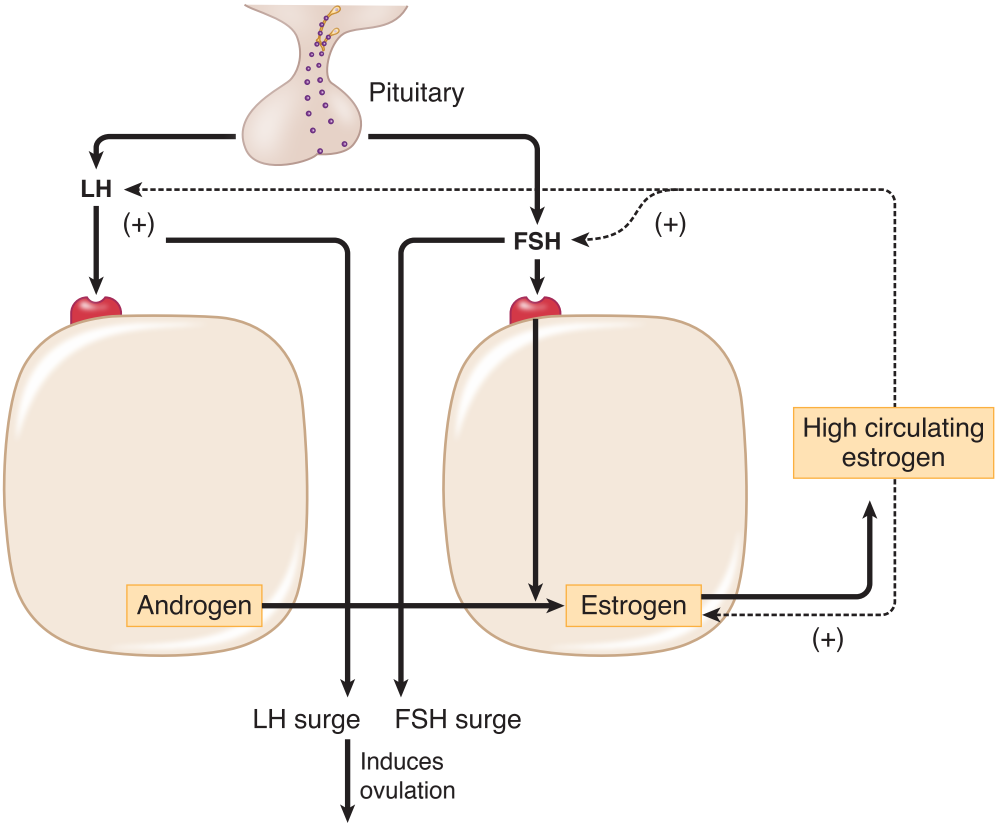


Figure VII-11-2. Pituitary-Ovarian Relationships at Ovulation

Estrogen Levels

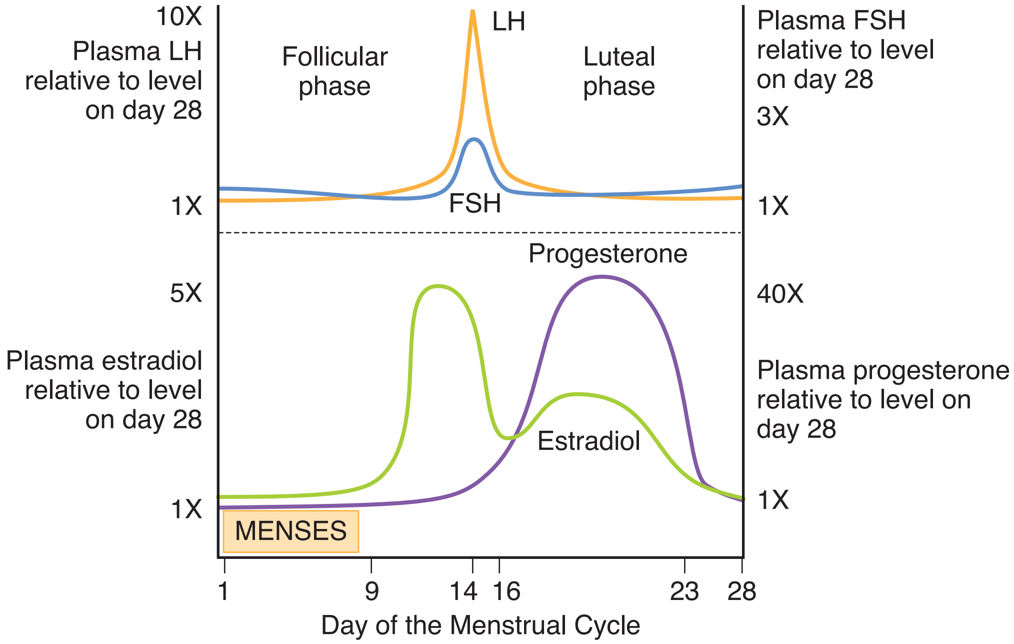
Near the end of the follicular phase, there is a dramatic rise in circulating estrogen. When estrogens rise above a certain level, they no longer inhibit the release of LH and FSH. Instead, they stimulate the release of LH and FSH (negative feedback loop to positive feedback loop).

This causes a surge in the release of LH and FSH. Only the LH surge is essential for the induction of ovulation and formation of the corpus luteum. Notice from the figure that the LH surge and ovulation occur after estrogen peaks. Therefore, if estrogens are still rising, ovulation has not occurred.

Follicular rupture occurs 24–36 hours after the onset of the LH surge. During this time interval, LH removes the restraint upon meiosis, which has been arrested in prophase for years. The first meiotic division is completed, and the first polar body is extruded.

Positive feedback loops are rare in the body. Ovulation with estrogen and parturition with oxytocin are examples of positive feedback loops.

Luteal phase (~days 14–28)



Luteinization of the preovulatory follicle

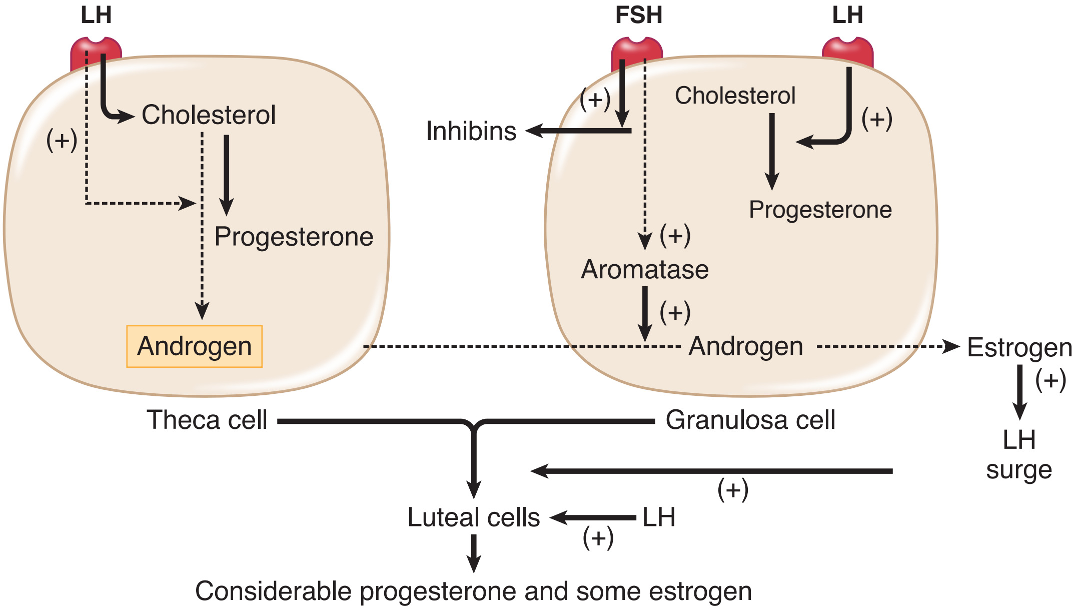


Figure VII-11-3. The Luteal Phase Reactions

Preovulatory Follicle

In the latter stages of the follicular phase, intracellular changes within the granulosa and theca cells occur in preparation for their conversion into luteal cells.

- Estradiol, in conjunction with FSH, causes the granulosa cells to produce LH receptors.
- The metabolic pathways are then altered to favor the production of progesterone.
- This would include a decrease in the activity of aromatase and a drop in estrogen production.

LH Surge

Induced by the elevated estrogens, it causes the granulosa cells and theca cells to be transformed into luteal cells and increases the secretion of progesterone.

Corpus Luteum

The process of luteinization occurs following the exit of the oocyte from the follicle. The corpus luteum is made up of the remaining granulosa cells, thecal cells, and supportive tissue. Once formed, the luteal cells are stimulated by LH to secrete considerable progesterone and some estrogen. Progesterone inhibits LH secretion (negative feedback). The corpus luteum secretes inhibin A, which has negative feedback on FSH.

The increased plasma level of progesterone has several actions:

- It causes the uterine endometrium to become secretory, providing a source of nutrients for the blastocyst.
- It causes the cervical mucus to become thick, sealing off the uterus from further entry of sperm or bacteria.
- It has thermogenic properties, causing the basal body temperature to increase by 0.5–1.0° F.

Menses

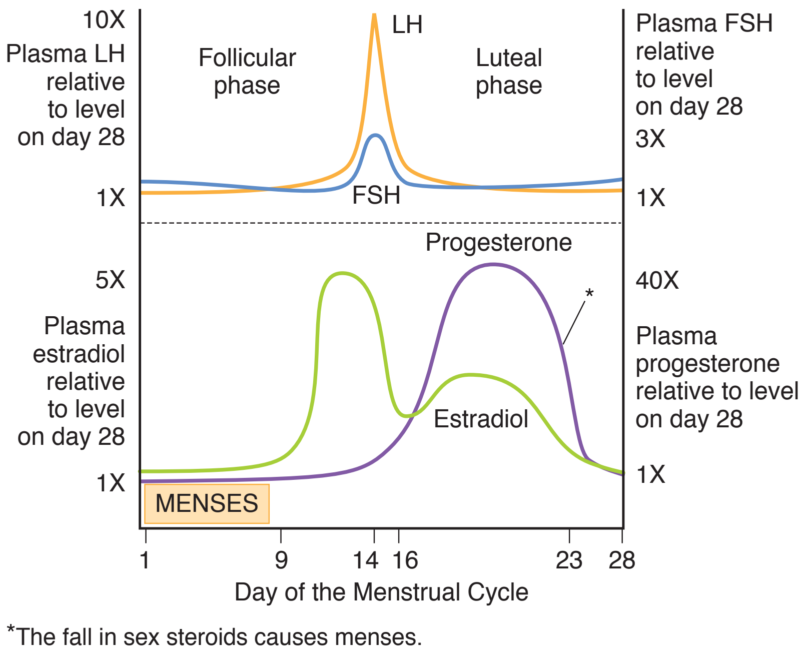


Figure VII-11-4. Onset of Menses

- The life of the corpus luteum is finite, hence the luteal phase is only 14 days.
- Initially, the corpus luteum is very responsive to LH. Over time however, as the corpus luteum becomes less functional, it becomes less responsive to LH.
- Progesterone exerts negative feedback on LH, which contributes to the demise of the corpus luteum.
- With the demise of the corpus luteum, progesterone and estradiol fall to levels that are unable to support the endometrial changes, and menses begins.

Menstruation is due to a lack of gonadal sex steroids.

FEMALE SEX STEROID METABOLISM AND EXCRETION

Solubilization and Excretion

The female sex steroids undergo oxidation or reduction in the liver (and other target tissues), and a glucuronide or sulfate group is attached to the steroidal metabolite. This “conjugation” increases the solubility of the steroids in water, and they thus become excretable in urine.

Estradiol can be excreted as a conjugate of estradiol, but most is first converted to estrone or estriol.

Progesterone is converted in the liver to pregnanediol and is excreted as pregnanediol glucuronide.

Monitoring the Menstrual Cycle

The amount of sex steroids excreted in the urine can be used to monitor the menstrual cycle. For example:

- Low progesterone metabolites and low but slowly rising estrogen metabolites characterize the early follicular phase.
- Low progesterone metabolites and rapidly rising estrogen metabolites characterize the latter part of the follicular phase just before ovulation.
- Elevated levels of progesterone metabolites characterize the luteal phase and pregnancy. In the early luteal phase progesterone is rising, in the latter half it is falling.

Estrogens and Androgen Formation

- Estrogen: generic term for any estrus-producing hormone, natural or synthetic
- 17 β-estradiol: major hormone secreted by the ovarian follicle
- Estrone: some is secreted from the ovary but much is formed in peripheral tissues such as adipose tissue from androgens. These androgens originate from both the ovary and the adrenal glands. This is the main circulating estrogen following menopause. Fat cells have aromatase. Adipose tissue creates modest levels of estrogen.
- Estriol: major estrogen synthesized from circulating androgens by the placenta
- Potency: estradiol > estrone > estriol
- Androgens: The follicles also secrete androgen; DHEA, androstenedione, and testosterone. Additional testosterone production is from the peripheral conversion of adrenal and ovarian androgen. Some testosterone is also converted via 5 α-reductase to dihydrotestosterone in the skin.

New Cycle

During the 3 days prior to and during menses, plasma levels of progesterone and estradiol are at their low point; negative feedback restraint for gonadotropin secretion is removed. FSH secretion rises slightly and initiates the next cycle of follicular growth.

The length of the follicular phase of the menstrual cycle is more variable than the length of the luteal phase. Long cycles are usually due to a prolonged follicular phase and short cycles to a short follicular phase. Once ovulation has occurred, menses generally follows in about 14 days. The length of the menstrual cycle in days minus 14 gives the most likely day of ovulation.

Recall Question

Which of the following is the physiologic cause of menstruation?

A. LH surge increasing the secretion of progesterone

B. Rising levels of estrogen causing endometrial cells of the uterine to proliferate

C. Withdrawal of hormonal support of the endometrium

D. LH removes the restraint on meiosis

E. Increase in plasma levels of progesterone

Answer: C

MENSTRUAL IRREGULARITIES

Amenorrhea

Amenorrhea is the lack of menstral bleeding. Though in itself it does not cause harm, it may be a sign of genetic, endocrine, or anatomic abnormalities.

- In the absence of anatomic abnormalities (and pregnancy), it usually indicates a disruption of the hypothalamic–pituitary axis or an ovarian problem.
- A hypothalamic–pituitary origin would include Kallman's syndrome, functional hypothalamic amenorrhea, amenorrhea in female athletes, eating disorders, hypothyroidism (possibly because high TRH stimulates prolactin), and pituitary tumors such as prolactinomas.
- Ovarian causes could be premature ovarian failure (premature menopause), repetitive ovulation failure, or anovulation (intermittent bleeding), or a polycystic ovary.

Polycystic Ovarian Syndrome

Polycystic ovarian syndrome is characterized by an elevated LH/FSH ratio.

- Clinical signs include infertility, hirsutism, obesity, insulin resistance, and amenorrhea and oligomenorrhea.
- The enlarged polycystic ovaries are known to be associated with increased androgen levels (DHEA).
- It originates in obese girls. The high extraglandular estrogens (mainly estrone) selectively suppress FSH. Ovarian follicles do have a suppressed aromatase activity and thus a diminished capacity to convert androgen into estrogen, but the adrenals may also contribute to the excess androgens as well.
- High androgens promote atresia in developing follicles and disrupt feedback relationships. Look for high LH and DHEA levels.
- The overall result is anovulation-induced amenorrhea with an estrogen-induced endometrial hyperplasia and breakthrough bleeding.

- Although poorly understood the hyperinsulinemia is believed to be a key etiologic factor.
- Treat amenorrhea in PCOS with metformin.
- Treat androgenization with spironolactone.

Hirsutism

Hirsutism is an excessive, generally male, pattern of hair growth. It is often associated with conditions of androgen excess, e.g., congenital adrenal hyperplasia and polycystic ovarian syndrome.

- Virilization refers to accompanying additional alterations, such as deepening of the voice, clitoromegaly, increased muscle bulk, and breast atrophy.
- Axillary and pubic hair are sensitive to low levels of androgen.
- Hair on the upper chest, face (scalp region not involved), and back requires more androgen and represents the pattern seen in males.
- Circulating androgens involved are testosterone, DHEA, DHEAS, and androstenedione in response to LH and ACTH.
- Measurements of DHEAS as well as a dexamethasone suppression test helps in separating an adrenal from an ovarian source.
- Polycystic ovarian syndrome is the most common cause of ovarian androgen excess.

PREGNANCY

Ovum Pickup and Fertilization

In women, the ovum is released from the rupturing follicle into the abdominal cavity, where it is “picked up” by the fimbria of the oviduct. Failure of ovum pickup may result in ectopic pregnancy, i.e., the implantation of the blastocyst at any site other than the interior of the uterus.

Fertilization occurs in the upper end of the oviduct within 8–25 hours after ovulation. After this, the ovum loses its ability to be fertilized. Sperm retain their capacity to fertilize an ovum for as long as 72 hours after ejaculation. For about 48 hours around the time of ovulation the cervical mucus is copious and slightly alkaline. This environment represent a good conduit for the sperm.

Weeks of gestation (gestational age) to estimate the delivery date are commonly taken from the first day of the last menstrual period.

Sperm are transported from the vagina to the upper ends of the oviduct by contraction of the female reproductive tract. The swimming motions of the sperm are important for penetration of the granulosa cell layer (cumulus oophorus) and membranes surrounding the ovum.

Low sperm counts (<20 million/mL of ejaculate) are associated with reduced fertility because sperm from ejaculates with low counts often contain many sperm with poor motility and an abnormal morphology. The first step in infertility evaluation is semen analysis.

Implantation

At the time of implantation, which occurs about 5-7 days after fertilization, the development is at the blastocyst stage. The trophoblastic cells of the fetus now begin to secrete a peptide hormone, human chorionic gonadotropin (hCG). HCG starts 10 days after fertilization.

Fetal hCG possesses a β subunit similar to that of LH, and therefore it has considerable LH activity.

The presence of the beta subunit of hCG in the urine can be detected by a variety of test kits for the detection of pregnancy.

Hormonal Maintenance of the Uterine Endometrium

The production of estrogen and progesterone during pregnancy can be divided into 3 phases:

- Part of the luteal phase before implantation
- Early pregnancy
- Late pregnancy

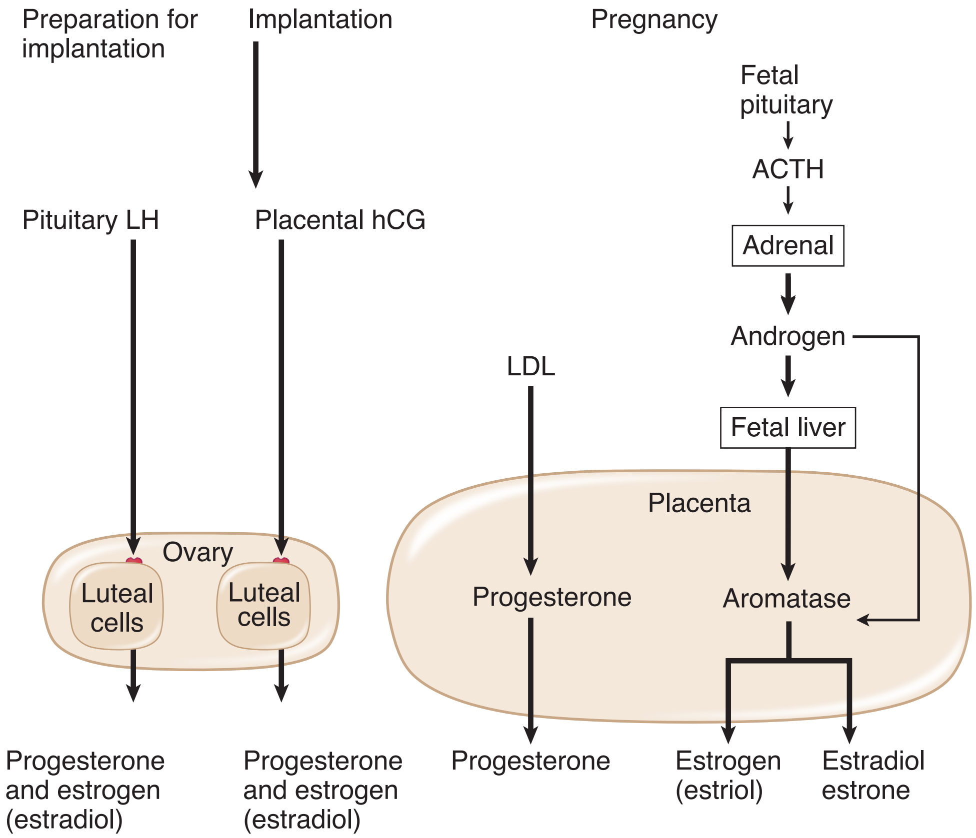


Figure VII-11-5. Steroids During Pregnancy

Preparation for implantation (luteal phase)

Pituitary LH stimulates luteal cells to secrete progesterone and some estrogen. Because the ovaries are the source of the estrogen, it is mainly estradiol.

Implantation to second month

- Within a week or two of fertilization, trophoblastic cells of the placenta begin secreting hCG. In short, hCG prevents regression of the corpus luteum, thus allowing it to continue producing estrogens and progesterone.
- hCG doubles in the early weeks of pregnancy. Because it maintains secretion of progesterone from the corpus luteum, progesterone is a sensitive marker of early fetal well-being.
- Loss of the corpus luteum during this period terminates the pregnancy. However, in lieu of the corpus luteum, exogenous progesterone would be a functional substitute.

Third month to term

- Placenta secretes enough progesterone and estrogen to maintain the uterus. This is not controlled by hCG. At this time, the ovaries (corpus luteum) can be removed and pregnancy continues.
- Progesterone secretion of the placenta is limited only by the amount of precursor (cholesterol) delivered by low-density lipoproteins (LDL) to the placenta. Progesterone maintains uterine quiescence during pregnancy.
- The secretion of estrogen involve both the fetus and the placenta.
- The fetal adrenal gland secretes dehydroepiandrosterone (DHEA). The fetal liver then converts DHEA to androstenedione (A) and testosterone.
- The placenta expresses aromatase. This enzyme converts the A and testosterone from the fetus into estrogens, estriol being the primary one. Thus, estriol becomes a good marker for fetal well-being.

Peripheral Effects of Hormonal Changes

The large amount of estrogen and progesterone secreted by the placenta during pregnancy stimulates the following important changes within the mother:

- Massive growth of the uterus, especially the myometrium
- Increased growth of all components (glands, stroma, and fat) of the breasts

Additional hormonal changes

Increased prolactin secretion by the pituitary in response to elevated estrogens

Secretion of human placental lactogen (hPL), also called human chorionic somatomammotropin (hCS), by the placenta. This markedly increases during the latter half of the pregnancy.

- hPL (hCS) has considerable amino acid sequence homology with growth hormone but has very little growth-stimulating activity.
- hPL (hCS) has metabolic actions similar to growth hormone; that is, it increases maternal lipolysis and ketogenesis and decreases maternal glucose utilization, thereby making maternal energy stores more available for the fetus.
- During the second trimester pregnancy becomes a hyperinsulinemic state with peripheral resistance to the metabolic effects of insulin. This reserves glucose for fetal needs and the mother depends more heavily on fatty acids as a source of energy. Under these conditions even modest fasting can cause ketosis.
- These anti-insulin actions of hPL (hCS) may also account for the gestational diabetes that develops in some pregnant women.
- hPL (hCS) is secreted in proportion to the size of the placenta and is an index of placental well-being.

PRL = prolactin
Prog = progesterone

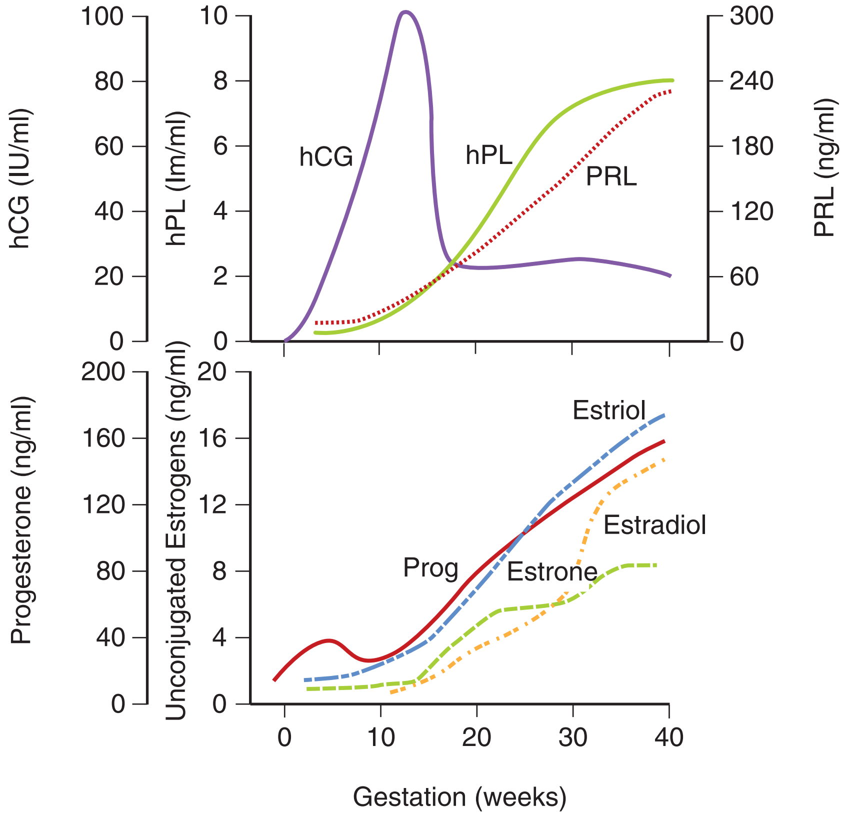


Figure VII-11-6. Hormone Levels During Pregnancy

Maternal Compensatory Changes of Pregnancy

Cardiovascular/renal

Cardiac output increases but peripheral resistance decreases and as a result there is no hypertension associated with a normal pregnancy (parallel circuit of placenta). Blood pressure declines in the first trimester and gradually rises toward prepregnancy levels thereafter.

GFR increases and renal threshold decreases. Combined with the increased plasma glucose, glucose often appears in the urine.

Endocrine

The anterior pituitary enlarges by about one-third, due to a hyperplasia of the lactotrophs driven by the rise in estrogen. Postpartum pituitary necrosis (Sheehan syndrome) is preceded by obstetric hemorrhage. The posterior pituitary is usually spared. Failure to lactate is the most common clinical sign. Other manifestations would include the consequences of hypothyroidism and hypocortisolism.

Estrogen increases the circulating steroid-binding globulins and bound hormone increases but FT_4 is normal. Hyperthyroidism increases the risk of preterm delivery. Hypothyroidism is unusual in pregnancy.

Estrogen increases renin secretion, and overall increased activity of the renin-angiotensin-aldosterone system causes fluid retention and hemodilution.

Changes induced near the end of pregnancy

The pubic symphysis, cervix, and vagina become more distensible. These changes make passage of the fetus through the birth canal easier. The peptide hormone relaxin, which is secreted by the placenta, also promotes these changes. Its action is not essential. Parturition in humans is normal in the absence of ovaries.

In response to elevated plasma estrogens, oxytocin receptors increase in the myometrium. Thus, the sensitivity of the uterine myometrium to the excitatory action of oxytocin is increased.

Parturition

The factors that initiate parturition are not well understood, but the following facts are known:

- As indicated above, estrogens and progesterone are high throughout pregnancy. Estrogens upregulate phospholipase A2 in the amnion as well as oxytocin receptors in the myometrium.
- Prostaglandins cause contraction of the uterus and are thought to initiate the labor process. Contraction of the myometrium pushes the fetus towards the cervix.

- Dilation of the cervix stimulates afferent neurons that cause the release of oxytocin from the posterior pituitary gland. Oxytocin contracts the myometrium and stimulates local production of prostaglandins. This contraction is thought to participate in parturition, and contraction of the uterus by oxytocin is thought to play an important role in limiting blood loss after the fetus is expelled.
- When a fetus dies, toxic products originating from the fetus increase prostaglandin release in the uterus, thus initiating contractions and a spontaneous abortion (miscarriage). Similarly, administration of prostaglandins induces abortion.

LACTATION

Mammary Gland Growth and Secretion

Growth of mammary tissue is stimulated by the female sex steroids estrogen and progesterone. However, for these steroids to stimulate maximum growth, prolactin, growth hormone, and cortisol also must be present.

During pregnancy, the high levels of plasma estrogen greatly increase prolactin secretion, but milk synthesis does not occur because the high level of estrogen (and progesterone) blocks milk synthesis. At parturition, plasma estrogen drops, withdrawing the block on milk synthesis. As a result, the number of prolactin receptors in mammary tissue increases several-fold, and milk synthesis begins.

Maintaining Lactation

Suckling is required to maintain lactation.

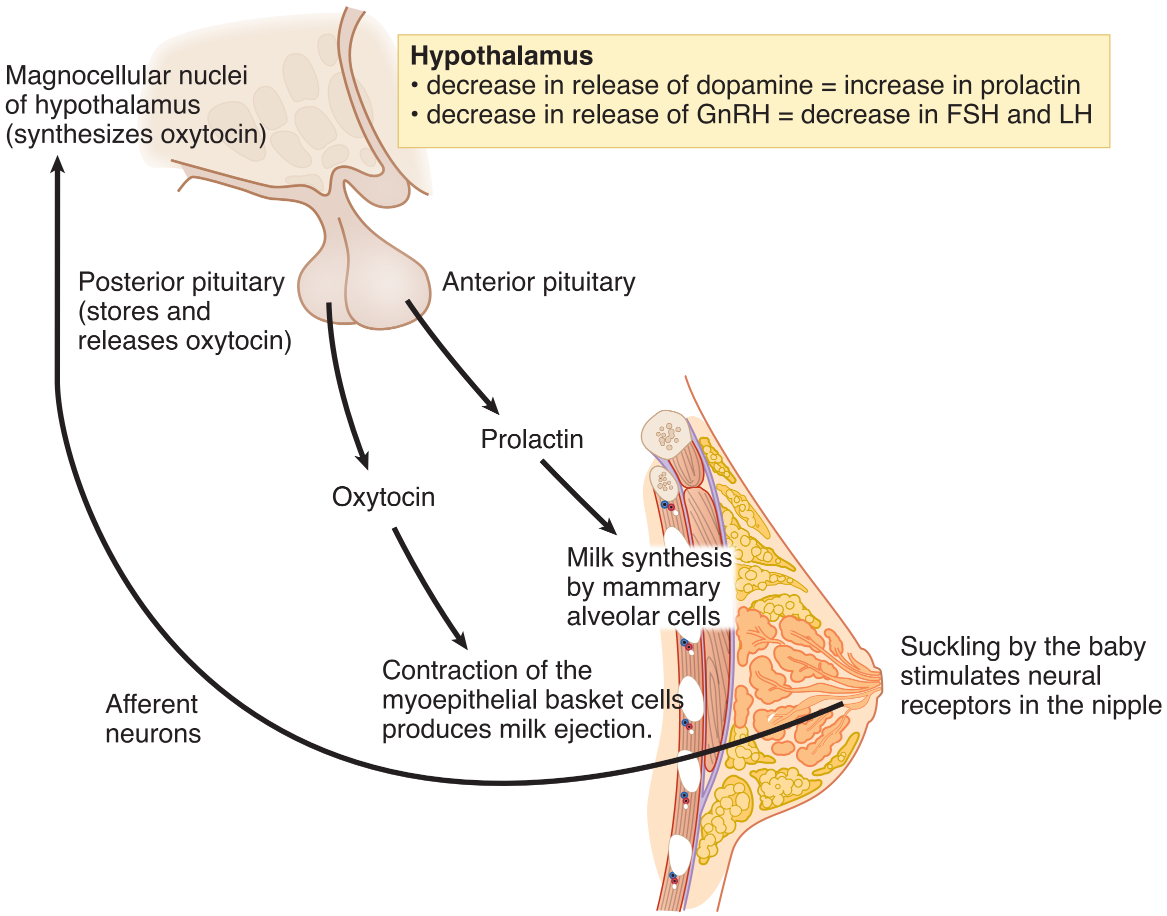


Figure VII-11-7. Lactation and the Suckling Reflex

The suckling of the baby at the mother's breast stimulates receptors in the mother's nipples. Signals from these receptors are transmitted to the hypothalamus and have the following effects:

- Oxytocin synthesis and secretion are increased. Oxytocin causes the myoepithelial basket cells that surround the alveoli to contract. Preformed milk is ejected into the ducts and out the openings of the nipple; that is, milk ejection is initiated.
- The release of dopamine by the hypothalamus into the hypophyseal portal vessels is inhibited. This removes a chronic restraint on prolactin secretion. Prolactin secretion increases, and milk secretion is stimulated each time the baby suckles.
- The secretion of GnRH into the hypophyseal portal vessels is inhibited; secretion of FSH and LH decreases. Thus, follicular growth, estrogen secretion, ovulation, and menses cease. High prolactin levels also contribute to the amenorrhea.

For the suckling stimulus to inhibit GnRH secretion completely, the stimulus must be prolonged and frequent. Supplementation of the mother's milk with other fluids or sources of energy reduces the baby's suckling and allows gonadotropin secretion, follicular growth, and ovulation to occur.

Women who do not wish to breastfeed their children are sometimes administered large doses of estrogen. The estrogen inhibits lactation (by its inhibitory action of milk synthesis), even though estrogen promotes increased prolactin secretion.

Breastfeeding is a form of contraceptive because it should stop ovulation.

PART VIII

Gastrointestinal Physiology

Overview and Motility 1

Learning Objectives

- ❑ Explain how the gastrointestinal tract is structured
- ❑ Explain nervous control and endocrine control of the gastrointestinal system
- ❑ Explain information related to motility

THE GASTROINTESTINAL TRACT

Structure

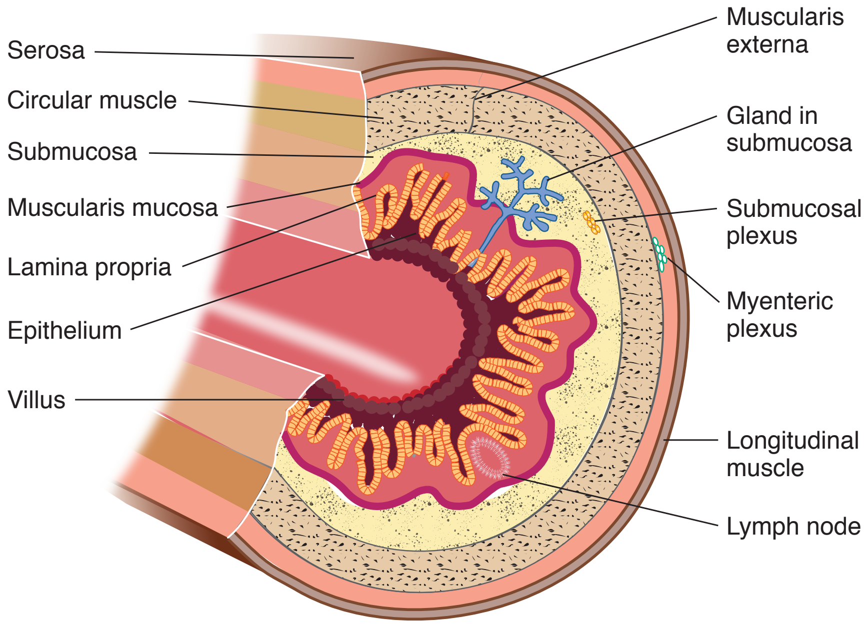


Figure VIII-1-1. Gastrointestinal (GI) Tract

Mucosa

- Epithelium: consists of a single layer of specialized cells; some are involved in secretions and some release hormones
- Lamina propria: layer of connective tissue which contains glands, hormone-containing cells, lymph nodes, and capillaries
- Muscularis mucosa: a thin layer of muscle, the contraction of which causes folding and ridges in the mucosal layers

Submucosa

- Layer of connective tissue that contains glands, large blood vessels, and lymphatics
- Outermost region has a nerve net called the submucosal (Meissner's) plexus; Meissner's plexus is part of the enteric nervous system and is involved in secretory activity

Muscularis externa

- Inner layer of circular muscle
- Outer layer of longitudinal muscle
- Myenteric nerve plexus involved in motor activity is between the muscle layers

Note

Sympathetic regulation of the splanchnic circulation does not involve the enteric nervous system.

Serosa

- Outermost layer of GI tract
- Consists of connective tissue and a layer of epithelial cells
- Within this layer autonomic nerve fibers run and eventually synapse on target cells and the enteric nerve plexes

Nervous Control

Residing in the GI tract is a vast neural network called the enteric nervous system (Meissner's and myenteric plexi). Normal GI function is dependent on this neural network. The enteric nervous system is innervated by the autonomic nervous systems and serves as the final mediator for virtually all neurally mediated changes.

Sympathetic

The diagram below illustrates how the synaptic junction at the end of a nerve fiber secretes norepinephrine (NE), which then induces responses in the gastrointestinal (GI) system.

———————— < NE

↓ motility
↓ secretions
↑ constriction of sphincters

An increase in sympathetic activity slows processes.

Parasympathetic

ACH		↑ motility ↑ secretions
VIP		↓ constriction of sphincters
GRP	G cell	↑ gastrin

Via the enteric nervous system, an increase in parasympathetic activity promotes digestive and absorptive processes.

> VIP: vasoactive intestinal peptide, released from enteric neurons
>
> GRP: gastrin-releasing peptide; stimulates the release of gastrin from G cells

Endocrine Control

Table VIII-1-1. Endocrine Control of GI System

Hormone**	Source	Stimulus	Stomach Motility and Secretion	Pancreas	Gallbladder
Secretin	S cells lining duodenum	Acid entering duodenum	Inhibits	Stimulates fluid secretion (HCO_3^-)	
CCK	Cells lining duodenum	Fat and amino acids entering duodenum	Inhibits emptying	Stimulates enzyme secretion	1. Contraction 2. Relaxation sphincter (Oddi)
Gastrin	G cells of stomach	Stomach distension	Stimulates		
	Antrum	Parasym (GRP) Peptides			
	Duodenum	Stomach acid inhibits*			
GIP **GLP**	Duodenum	Fat, CHO, amino acids	Inhibits	Increases insulin Decreases glucagon	

CCK = cholecystokinin; GIP = gastric inhibitory peptide (glucose insulinotropic peptide), GLP = glucagon-like peptide

*Note: In a non–acid-producing stomach (e.g., chronic gastritis), the reduced negative feedback increases circulating gastrin.

**All 4 hormones stimulate insulin release.

MOTILITY

Characteristics of Smooth Muscle

Electrical activity

- Resting membrane potential –40 to –65 mV. Close to depolarization.
- Oscillation of membrane potential is generated by interstitial cells (interstitial cells of Cajal) that act as pacemakers. This is referred to as slow waves or basic electrical rhythm, and if threshold is reached it generates action potentials.
- Action potentials are generated by the opening of slow channels that allow the entry of both sodium and calcium.
- The duodenum contracts the most often.

Note

Anticholinergic medications such as atropine or tricyclic antidepressants slow GI motility.

Note

Nerve gas increases GI and bronchial secretions.

Motor activity

- Stretch produces a contractile response.
- Gap junctions create an electrical syncytium within the smooth muscle.
- Slow waves create low level contractions, and action potentials strengthen the contractions.
- Pacemaker activity from the interstitial cells creates the intrinsic motor activity.
- Tonic contraction at sphincters act as valves.

Swallowing

Swallowing is a reflex controlled from the brain stem. Efferent input is via the vagus nerve for all events.

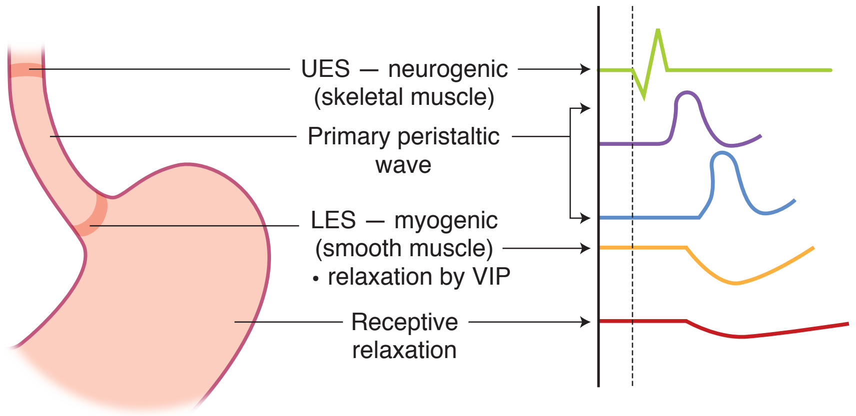


Figure VIII-1-2. Swallowing, the Peristaltic Wave

Note

Sympathetic slows parasympathetic speeds.

An increase in sympathetic activity slows processes.

Events during swallowing:

- Relaxation of upper esophageal skeletal muscle sphincter (UES)
- Primary peristaltic wave
- Relaxation of lower esophageal smooth muscle sphincter (LES) via VIP, which relaxes smooth muscle via NO
- Relaxation of proximal stomach (receptive relaxation)

Bridge to Pathology

Barrett esophagus is the term used to describe alterations in the esophageal epithelium that accompany GERD.

If the primary peristaltic wave is not successful, a secondary peristaltic wave is initiated by local distension of the esophagus. The secondary wave is not “conscious.”

Disorders of the Esophagus

Achalasia

- Failure of the LES to relax, resulting in swallowed food being retained in the esophagus
- Caused by abnormalities in the enteric nerves
- Peristaltic waves are weak

Gastroesophageal reflux disease (GERD)

- LES doesn't maintain tone
- Acid reflux damages esophageal epithelium

Diffuse esophageal spasm

- Spasms of esophageal muscle
- Presents with characteristics of a heart attack (e.g., chest pain)
- Barium swallow shows repeated, spontaneous waves of contraction

Note

Dysphagia refers to difficulty in swallowing.

Gastric Motility

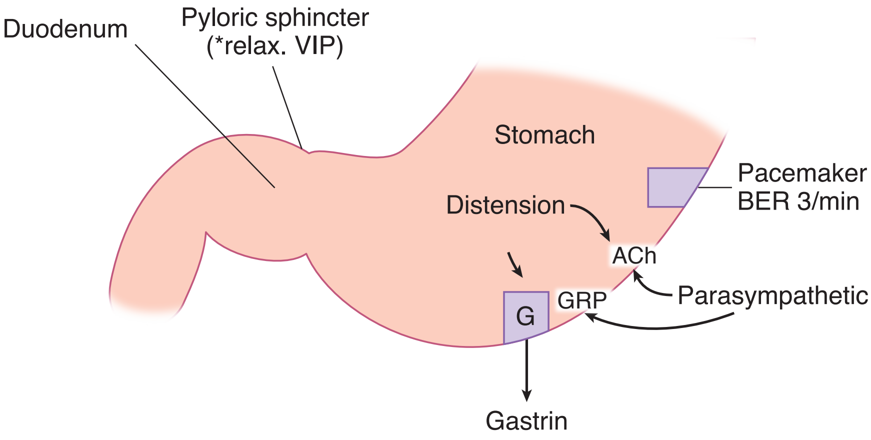


Figure VIII-1-3. Endocrine and Neural Control of the Stomach

ACh: acetylcholine

BER: basic electrical rhythm

GRP: gastrin-releasing peptide

VIP: vasoactive intestinal polypeptide

Stimulation

- Acetylcholine released in response to activation of parasympathetics
- Local distension

Inhibition

- Low pH of stomach contents inhibits the release of gastrin
- Feedback from duodenal release of hormones (CCK, secretin, and GIP)

Stomach emptying

- Liquids > CHO > protein > fat (> = faster than)
- The pyloris of the stomach acts as a sphincter to control the rate of stomach emptying. A wave of contraction closes the sphincter so that only a small volume is moved forward into the duodenum. CCK, GIP, and secretin increase the degree of pyloric constriction and slow stomach emptying.

Small Intestinal Motility

Rhythmic contractions in adjacent sections create segmentation contractions, which are mixing movements. Waves of contractions preceded by a relaxation of the muscle (peristaltic movements) are propulsive.

- Ileocecal sphincter, or valve between the small and large intestine, is normally closed
- Distension of ileum creates a muscular wave that relaxes the sphincter
- Distension of colon creates a nervous reflex to constrict the sphincter

Colon Motility

Segmentation contractions create bulges (haustrations) along the colon. Mass movements, which are propulsive, are more prolonged than the peristaltic movements of the small intestine.

Migrating Motor Complex

Migrating motor complex (MMC) is a propulsive movement initiated during fasting. It begins in the stomach and moves undigested material from the stomach and small intestine into the colon.

During fasting, MMC repeats every 90–120 minutes. When one movement reaches the distal ileum, a new one starts in the stomach.

- Correlated with high circulating levels of motilin, a hormone of the small intestine
- Removes undigested material from the stomach and small intestine, and helps reduce bacterial migration from colon into the small intestine

Defecation

Defecation is a reflex involving the central nervous system. A mass movement in the terminal colon fills the rectum, causing a reflex relaxation of the internal anal sphincter and a reflex contraction of the external anal sphincter.

- Voluntary relaxation of the external sphincter accompanied with propulsive contraction of the distal colon complete defecation.
- Lack of a functional innervation of the external sphincter causes involuntary defecation when the rectum fills.

Secretions 2

Learning Objectives

- ❏ Demonstrate understanding of salivary, gastric, and pancreatic secretions
- ❏ Demonstrate understanding of the composition and formation of bile

SECRETIONS

Salivary Secretions

Parotid gland secretions are entirely serous (lack mucin). Submandibular and sublingual gland secretions are mixed mucus and serous. They are almost entirely under the control of the parasympathetic system, which promotes secretion.

The initial fluid formation in the acinus is via an indirect chloride pump (secondary active transport powered by the Na/K ATPase pump), and the electrolyte composition is isotonic and similar to interstitial fluid.

Duct cells modify the initial acinar secretion.

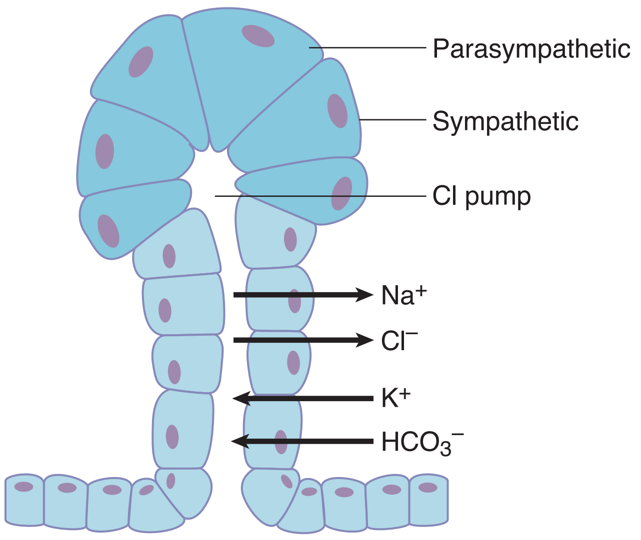


Figure VIII-2-1. Salivary Secretion

Composition of salivary secretions

- Low in Na^+, Cl^- because of reabsorption
- High in K^+, HCO_3 because of secretion (pH = 8)
- Low tonicity: Salivary fluid is hypotonic because of reabsorption of NaCl and impermeability of ducts to water.
- α-amylase (ptyalin): secreted in the active form and begins the digestion of carbohydrates
- Mucus, glycoprotein
- Immunoglobulins and lysozymes

Gastric Secretions

The epithelial cells that cover the gastric mucosa secrete a highly viscous alkaline fluid (mucin plus bicarbonate) that protects the stomach lining from the caustic action of HCl.

- Fluid needs both mucin and bicarbonate to be protective.
- Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin decrease the secretion of the mucin and bicarbonate.
- Surface of the mucosa studded with the openings of the gastric glands
- Except for the upper cardiac region and lower pyloric region whose glands secrete mainly a mucoid fluid, gastric glands secrete a fluid whose pH can be initially as low as 1.0.

Secretions of the main cells composing the oxyntic gastric glands

Parietal cells

- HCl
- Intrinsic factor combines with vitamin B_{12} and is reabsorbed in the distal ileum. This is the only substance secreted by the stomach that is required for survival. It is released by the same stimuli that release HCl.

Chief Cells

Pepsinogen is converted to pepsin by H^+, as illustrated in the diagram below.

$$\text{Pepsinogen} \xrightarrow{H^+} \text{pepsin (proteins to peptides)}$$

- Pepsinogen is initially converted to active pepsin by acid.
- Active pepsin continues the process.
- Pepsin is active only in the acid pH medium of the stomach.
- Pepsin begins the digestion of protein but is not essential for life.

Mucous Neck Cells

Mucous neck cells secrete the protective mucus, HCO_3 combination.

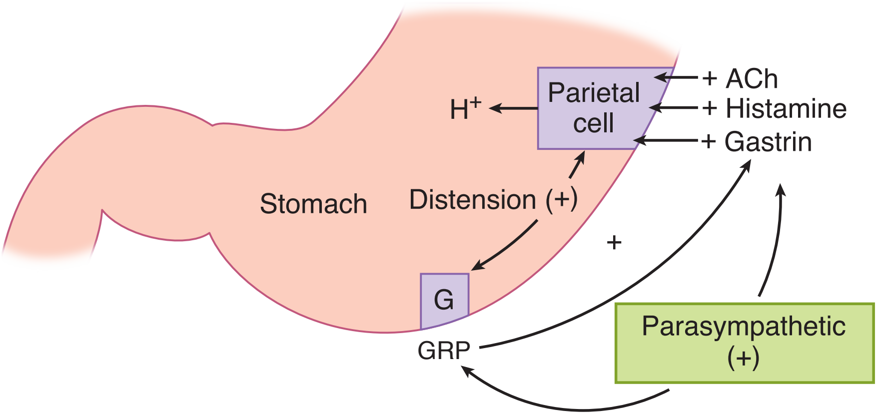


Figure VIII-2-2. Control of Gastric Acid Secretion

+ Stimulates secretion
– Inhibits secretion

Control of acid secretion

There are 3 natural substances which stimulate parietal cells (figure above):

- Acetylcholine (ACh), acting as a transmitter; release is stimulated by sight/smell of food and reflexly in response to stomach distension (vagovagal reflex).
- Locally released histamine; stimulated by Ach and gastrin
- The hormone gastrin; stimulated by release of GRP

As stomach pH falls, somatostatin (SST) is released, which inhibits gastrin and reduces acid secretion (feedback regulation of acid secretion).

Cellular mechanisms of acid secretion (figure below)

- Within the cell, carbonic anhydrase facilitates the conversion of CO_2 into H^+ and HCO_3^-.
- The demand for CO_2 can be so great following a meal that the parietial cells extract CO_2 from the arterial blood. This makes gastric venous blood the most basic in the body.
- Hydrogen ions are secreted by a H/K-ATPase pump similar to that in the distal nephron.
- The pumping of H^+ raises intracellular HCO_3^- and its gradient across the basal membrane and provides the net force for pumping Cl^- into the cell.
- The chloride diffuses through channels across the apical membrane, creating a negative potential in the stomach lumen.
- Because of the extraction of CO_2 and secretion of HCO_3^- , the venous blood leaving the stomach following a meal is alkaline.

- Compared with extracellular fluid, gastric secretions are high in H^+, K^+, Cl^-, but low in Na^+.
- The greater the secretion rate, the higher the H^+ and the lower the Na^+.
- Vomiting stomach contents produces a metabolic alkalosis and a loss of body potassium (hypokalemia mainly due to the alkalosis effect on the kidney).

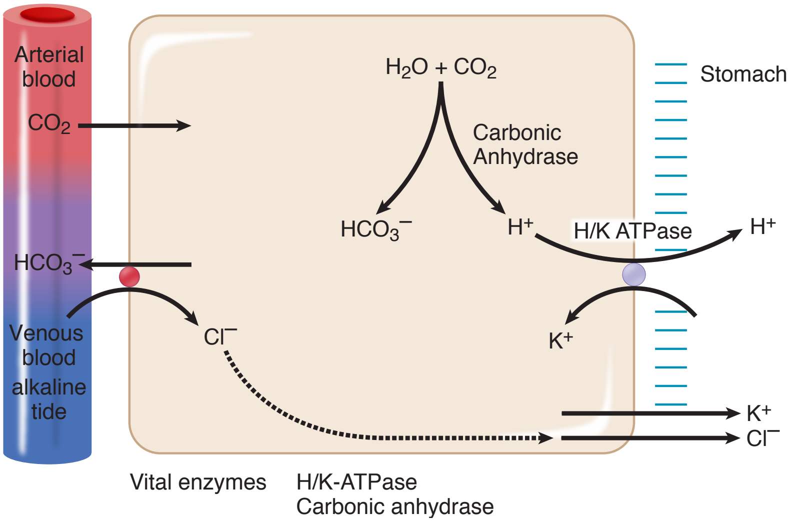


Figure VIII-2-3. Regulation of Parietal Cell Secretion

Pancreatic Secretions

Exocrine tissue is organized into acini and ducts very similar to that of the salivary glands.

- Cholinergic nerves to the pancreas stimulate the secretion of both the enzyme and aqueous component.
- Food in the stomach stimulates stretch receptors and, via vagovagal reflexes, stimulates a small secretory volume.
- Sympathetics inhibit secretion but are a minor influence.
- **Most of the control is via secretin and CCK.**

Enzymatic components

- Trypsin inhibitor, a protein present in pancreatic secretions, prevents activation of the proteases within the pancreas.
- In addition to the following groups of enzymes, pancreatic fluid contains ribonucleases and deoxyribonucleases.
- A diet high in one type of food (protein, CHO, fat) results in the preferential production of enzymes for that particular food.

Pancreatic amylases are secreted as active enzymes:

- Hydrolyze a-1,4-glucoside linkage of complex carbohydrates, forming three smaller compounds:
  - α-Limit dextrins: still a branched polysaccharide
  - Maltotriose, a trisaccharide
  - Maltose, a disaccharide
- Cannot hydrolyze β linkages of cellulose

Pancreatic lipases are mainly secreted as active enzymes. Glycerol ester lipase (pancreatic lipase) needs colipase to be effective. Colipase displaces bile salt from the surface of micelles. This allows pancreatic lipase to attach to the droplet and digest it, leading to formation of 2 free fatty acids and one monoglyceride (a 2-monoglyceride, i.e., an ester on carbon 2).

Cholesterol esterase (sterol lipase) hydrolyzes cholesterol esters to yield cholesterol and fatty acids. **Pancreatic proteases** are secreted as inactive zymogens. They include trypsinogen, chymotrypsinogen, and procarboxypeptidase.

Activation sequence. The activation sequences are summarized below.

trypsinogen $\xrightarrow{\text{enterokinase*}}$ trypsin (endopeptidase)

chymotrypsinogen $\xrightarrow{\text{trypsin}}$ chymotrypsin (endopeptidase)

procarboxypeptidase $\xrightarrow{\text{trypsin}}$ carboxypeptidase (exopeptidase)

*Enterokinase (also known as enteropeptidase) is an enzyme secreted by the lining of the small intestine. It is not a brush border enzyme. It functions to activate some trypsinogen, and the active trypsin generated activates the remaining proteases.

Fluid and electrolyte components

- Aqueous component is secreted by epithelial cells which line the ducts.
- Fluid is isotonic due to the high permeability of the ducts to water and the concentrations of Na and K are the same as plasma.
- Duct cells secrete chloride into the lumen via the cystic fibrosis transmembrane conductance regulator (CFTR). This chloride is then removed from the lumen in exchange for bicarbonate. Thus, bicarbonate secretion is dependent upon chloride secretion.
- CFTR is activated by cAMP (see below).
- In cystic fibrosis there is a mutation in the gene that encodes this CFTR channel, resulting in less chloride and a reduced fluid component of pancreatic secretions. The smaller volume of highly viscous fluid may also contain few enzymes.

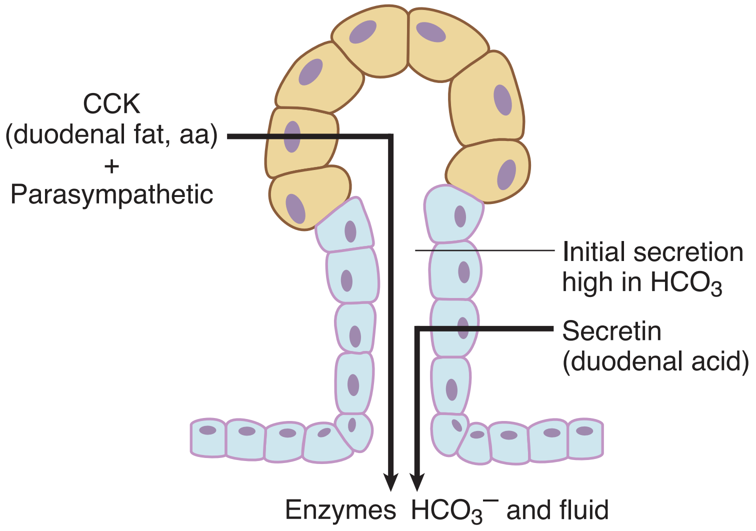


Figure VIII-2-4. Control of the Exocrine Pancreas

Control of pancreatic secretions

Most of the regulation is via 2 hormones: secretin and cholecystokinin.

Secretin is released from the duodenum in response to acid entering from the stomach.

- Action on the pancreas is the release of fluid high in HCO_3^-. Secretin is a peptide hormone that stimulates chloride entry into the lumen from duct cells. Secretin activates Gs–cAMP, which in turn activates CFTR.
- This released HCO_3^--rich fluid is the main mechanism that neutralizes stomach acid entering the duodenum.

Cholecystokinin (CCK) is released from the duodenum in response to partially digested materials (e.g., fat, peptide, and amino acids).

- Action on the pancreas is the release of enzymes (amylases, lipases, proteases).

Recall Question

Which of the following is a characteristic of GERD?

A. Failure of lower esophageal sphincter to relax

B. Odynophagia

C. Failure of lower esophageal sphincter to maintain its tone

D. Spasms of esophageal muscle

E. Presents with chest pain

Answer: C

COMPOSITION AND FORMATION OF BILE

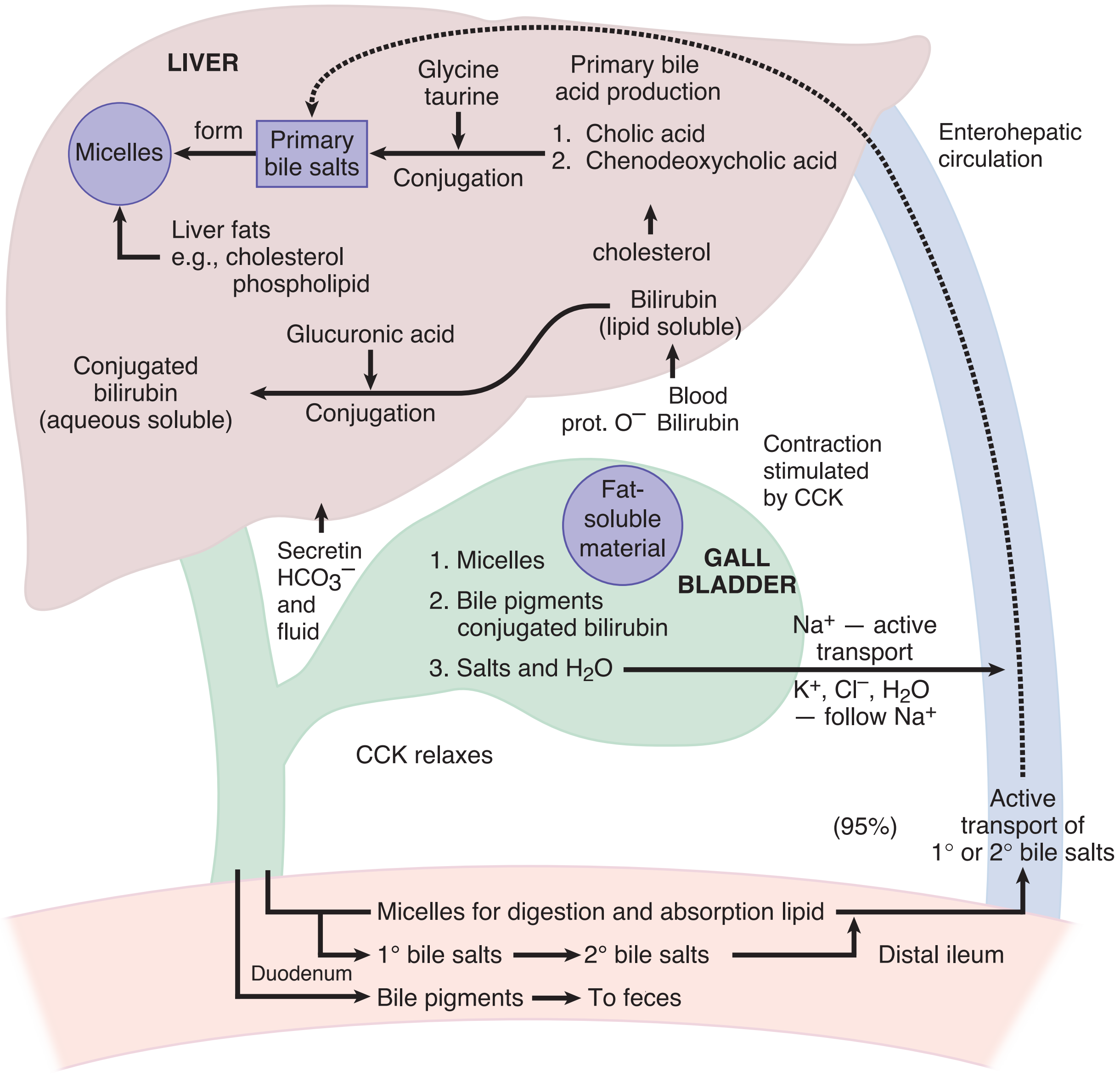


Figure VIII-2-5. Production and Metabolism of Bile

Bile salts and micelles

Primary bile acids known as cholic acid and chenodeoxycholic acid are synthesized by the liver from cholesterol.

- The lipid-soluble bile acids are then conjugated primarily with glycine.
- The conjugated forms are water-soluble but contain a lipid-soluble segment.
- Because they are ionized at neutral pH, conjugated bile acids exist as salts of cations (Na^+) and are, therefore, called bile salts.
- Bile salts are actively secreted by the liver.

- Secondary bile acids are formed by deconjugation and dehydroxylation of the primary bile salts by intestinal bacteria, forming deoxycholic acid (from cholic acid) and lithocholic acid (from chenodeoxycholic acid).
- Lithocholic acid has hepatotoxic activity and is excreted.
- When bile salts become concentrated, they form micelles. These are water-soluble spheres with a lipid-soluble interior.
- As such, they provide a vehicle to transport lipid-soluble materials in the aqueous medium of the bile fluid and the small intestine.
- Micelles are vital in the digestion, transport, and absorption of lipid-soluble substances from the duodenum to the distal ileum.
- In the distal ileum, and only in the distal ileum, can the bile salts be actively reabsorbed and recycled (enterohepatic circulation).
- Lack of active reabsorbing mechanisms (or a distal ileal resection) causes loss in the stool and a general deficiency in bile salts, as the liver has a limited capacity to manufacture them.
- This deficiency can lead to fat malabsorption and cholesterol gallstones.

Bridge to Pathology

Increased levels of plasma bilirubin produce jaundice. If severe, bilirubin can accumulate in the brain, producing profound neurological disturbances (kernicterus).

Bile pigments

A major bile pigment, **bilirubin** is a lipid-soluble metabolite of hemoglobin. Transported to the liver attached to protein, it is then conjugated and excreted as water-soluble glucuronides. These give a golden yellow color to bile.

Stercobilin is produced from metabolism of bilirubin by intestinal bacteria. It gives a brown color to the stool.

Salts and water

The HCO_3^- component is increased by the action of secretin on the liver.

The active pumping of sodium in the gallbladder causes electrolyte and water reabsorption, which concentrates the bile.

Bile pigments and bile salts are not reabsorbed from the gallbladder.

Phospholipids (mainly lecithin)

Insoluble in water but are solubilized by bile salt micelles

Cholesterol

Present in small amounts. It is insoluble in water and must be solubilized by bile salt micelles before it can be secreted in the bile.

Control of bile secretion and gallbladder contraction

- Secretin causes secretion of HCO_3– and fluid into bile canalicular ducts.
- Secretion of bile salts by hepatocytes is directly proportional to hepatic portal vein concentration of bile salts.
- CCK causes gallbladder contraction and sphincter of Oddi relaxation.

Enterohepatic circulation

- The distal ileum has high-affinity uptake of bile acids/salt (symport with Na^+).
- These bile acids/salts enter the portal vein and travel to the liver, which in turn secretes them into the cystic duct, from which they re-enter the duodenum.
- This recycling occurs many times during the digestion of a meal and plays a significant role in fat digestion.
- The synthesis of bile acids by the liver is directly related to the concentration of bile acids in the portal vein.

Small Intestinal Secretions

The most prominent feature of the small intestine is the villi.

- Surface epithelial cells display microvilli.
- Water and electrolyte reabsorption greatest at the villus tip.
- Water and electrolyte secretion greatest at the bottom in the crypts of Lieberkuhn.

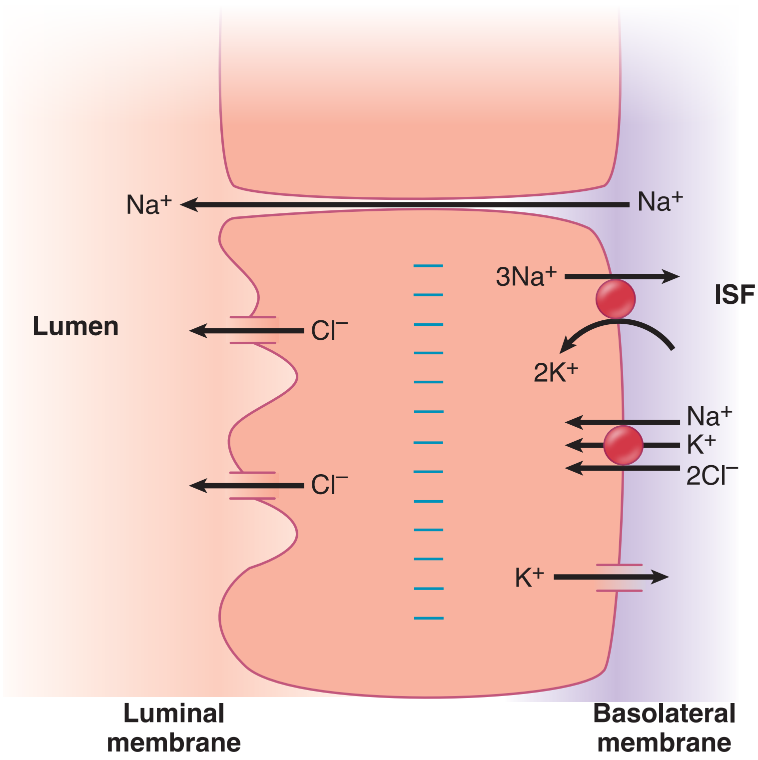


Figure VIII-2-6. Secretion of Electrolytes by a Crypt Cell of the Small Intestine

Bridge to Pathology

Cholera toxin binds and activates Gs, resulting in very high levels of intracellular cAMP. This rise in cAMP opens luminal Cl^- channels, causing a massive secretory diarrhea.

Crypt secretion

- A Na^+-K^+-$2Cl^-$ transporter in the basolateral membrane facilitates the ion uptake by secondary active transport.
- Na^+ entry drives the entry of K^+ and Cl^- into the cell.
- The elevated intracellular Cl and negative intracellular potential drives the diffusion of chloride through channels on the apical membrane.
- Luminal Cl then pulls water, Na, and other ions into the lumen, creating the isotonic secretion. This is the general scheme of the chloride pump.
- Neurotransmitter secretagogues include VIP and ACh.
- The Cl^- channels are opened by increases in cytosolic Ca^{2+} and/or cAMP. The cAMP-dependent Ca^{2+} channels are CFTR channels.

Digestion and Absorption 3

Learning Objectives

- ❏ Demonstrate understanding of digestion
- ❏ Answer questions about digestive enzymes and end products
- ❏ Demonstrate understanding of absorption

DIGESTION

The figure below summarizes the regional entry of the major digestive enzymes proceeding from the mouth, stomach, and through the small intestine.

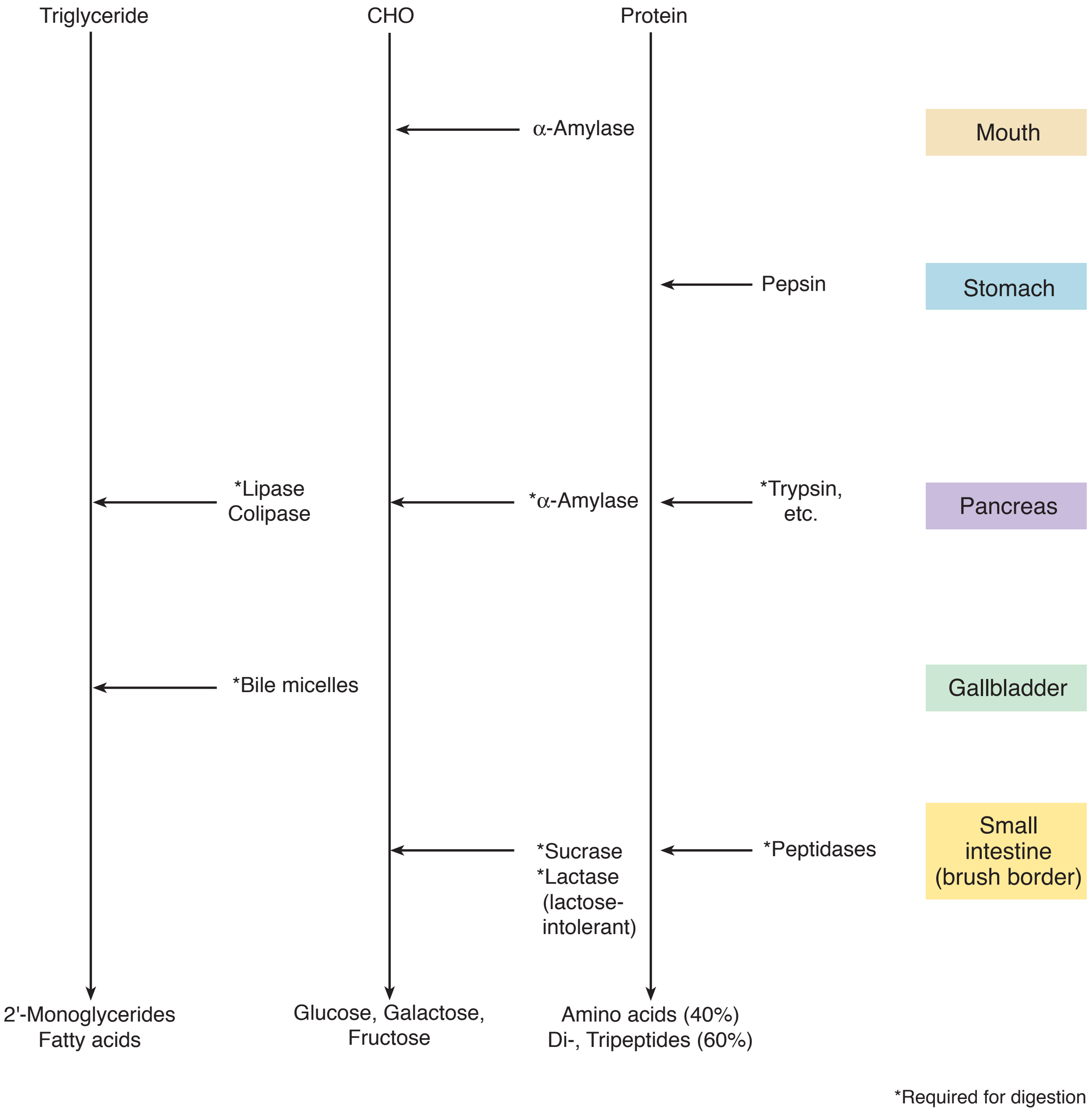


Figure VIII-3-1. Digestive Processes

Digestive Enzymes and End Products

Triglycerides

Stomach: Fatty materials are pulverized to decrease particle size and increase surface area.

Small intestine: Bile micelles emulsify the fat, and pancreatic lipases digest it. Micelles and pancreatic lipase are required for triglyceride digestion. The major end products are 2-monoglycerides and fatty acids.

Carbohydrates

Mouth: Salivary α-amylase begins the digestion, and its activity continues in the stomach until acid penetrates the bolus; however, it is not a required enzyme.

Small intestine: Pancreatic α-amylase, a required enzyme for CHO digestion, continues the process. α-amylase hydrolyzes interior bonds to produce oligosaccharides (limited dextrins) and disaccharides.

Brush border enzymes (sucrase-isomaltase; maltase; lactase; trehalase) convert limited dextrans and disaccharides into monosaccharides. These monosaccharides are then absorbed (late duodenum and early jejunum) via the mechanisms shown in the figure below.

Proteins

Stomach: Pepsin begins the digestion of protein in the acid medium of the stomach; however, it is not an essential enzyme.

Small intestine: Digestion continues with the pancreatic proteases (trypsin, chymotrypsin, elastase, and carboxypeptidases A and B), which are essential enzymes.

Protein digestion is completed by the small intestinal brush border enzymes, dipeptidases, and an aminopeptidase. The main end products are amino acids (40%) and dipeptides and tripeptides (60%).

Pancreatic enzymes are required for triglyceride, CHO, and protein digestion. Circulating CCK is almost totally responsible for their secretion following a meal.

ABSORPTION

Carbohydrate and Protein

The figure below illustrates the major transport processes carrying sugars and amino acids across the luminal and basal membranes of cells lining the small intestine.

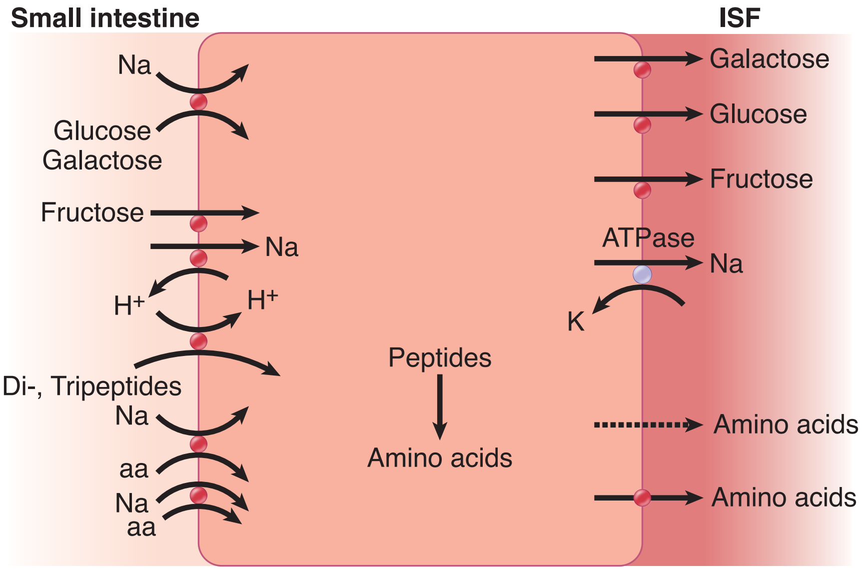


Figure VIII-3-2. Absorption of Carbohydrates and Proteins

Bridge to Pathology

Celiac disease is an immune reaction to gluten (protein found in wheat) that damages intestinal cells; the end result is diminished absorptive capacity of the small intestine.

Bridge to Pathology

Many of the amino acid transporters are selective for specific amino acids. Hartnup's disease is a genetic deficiency in the transporter for tryptophan.

Carbohydrate

- **Luminal membrane:** Glucose and galactose are actively absorbed (secondary active transport linked to sodium) via the sodium-glucose linked transporter 1 (SGLT-1). Fructose is absorbed independently by facilitated diffusion.
- **Basal membrane:** The monosaccharides are absorbed passively mainly via facilitated diffusion.

Protein

- **Luminal membrane:** amino acids are transported by secondary active transport linked to sodium. Small peptides uptake powered by a Na-H antiporter.
- **Basal membrane:** simple diffusion of amino acids, although it is now known some protein-mediated transport also occurs.

Lipids

The figure below summarizes the digestion and absorption of lipid substances. The end products of triglyceride digestion, 2-monoglycerides and fatty acids, remain as lipid-soluble substances that are then taken up by the micelles.

Digestive products of fats found in the micelles and absorbed from the intestinal lumen may include:

- Fatty acids (long chain)
- 2-monoglyceride
- Cholesterol
- Lysolecithin
- Vitamins A, D, E, K
- Bile salts, which stabilize the micelles

Micelles diffuse to the brush border of the intestine, and the water-soluble exterior allows them to carry fat soluble products into the cell.

In the mucosal cell, triglyceride is resynthesized and forms lipid droplets (chylomicrons). These leave the intestine via the lymphatic circulation (lacteals). They then enter the bloodstream via the thoracic duct.

The more water-soluble short-chain fatty acids can be absorbed by simple diffusion directly into the bloodstream. The bile salts are actively reabsorbed in the distal ileum.

Bridge to Biochemistry

Chylomicrons contain apolipoprotein B-48. Once in the systemic circulation, chylomicrons are converted to VLDL (very low-density lipoprotein) and they incorporate apoproteins C-II and E from HDL (high-density liproprotein).

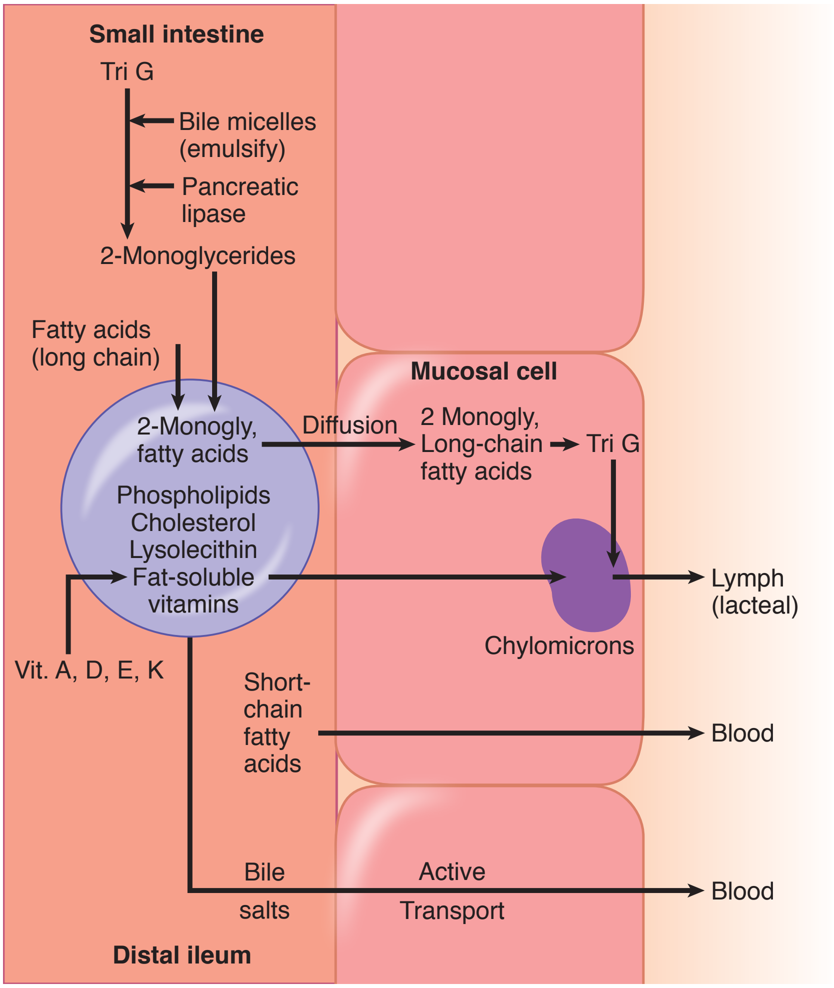


Figure VIII-3-3. Absorption of Lipids

Electrolytes

The net transport of electrolytes along the length of the small and large intestine is summarized in the figure below.

Duodenum

- Hypertonic fluid enters this region, and following the movement of some water into the lumen, the fluid becomes and remains isotonic (see crypt secretion above).
- The absorption of most divalent ions and water-soluble vitamins begins here and continues through the small intestine.

- Ingested iron and calcium tend to form insoluble salts. The acid environment of the stomach redissolves these salts, which facilitates their absorption in the small intestine. Iron and calcium absorption is diminished in individuals with a deficient stomach acid secretion.
- Calcium absorption is enhanced by the presence of calbindin in intestinal cells, and calcitriol (active vitamin D) induces the synthesis of this protein.
- Intestinal cells express the protein ferritin, which facilitates iron absorption.

Jejunum

- Overall, there is a net reabsorption of water and electrolytes.
- The cellular processes involved are almost identical to those described in the renal physiology section for the cells lining the nephron proximal tubule.

Ileum

- Net reabsorption of water, sodium, chloride, and potassium continues, but there begins a net secretion of bicarbonate.
- It is in the distal ileum, and only in the distal ileum, where the reabsorption of bile salts and intrinsic factor with vitamin B_{12} takes place.

Colon

- The colon does not have digestive enzymes or the protein transporters to absorb the products of carbohydrate and protein digestion.
- Also, because bile salts are reabsorbed in the distal ileum, very few lipid-soluble substances are absorbed in the colon.
- There is a net reabsorption of water and sodium chloride, but there are limitations.
- Most of the water and electrolytes must be reabsorbed in the small intestine, or the colon becomes overwhelmed.
- Most of the water and electrolytes are absorbed in the ascending and transverse colon; thereafter, the colon has mainly a storage function.
- The colon is a target for aldosterone, where it increases sodium and water reabsorption and potassium secretion.
- Because there is a net secretion of bicarbonate and potassium, diarrhea usually produces a metabolic acidosis and hypokalemia. It commonly presents as hyperchloremic, nonanion gap metabolic acidosis, as described in the acid-base section.

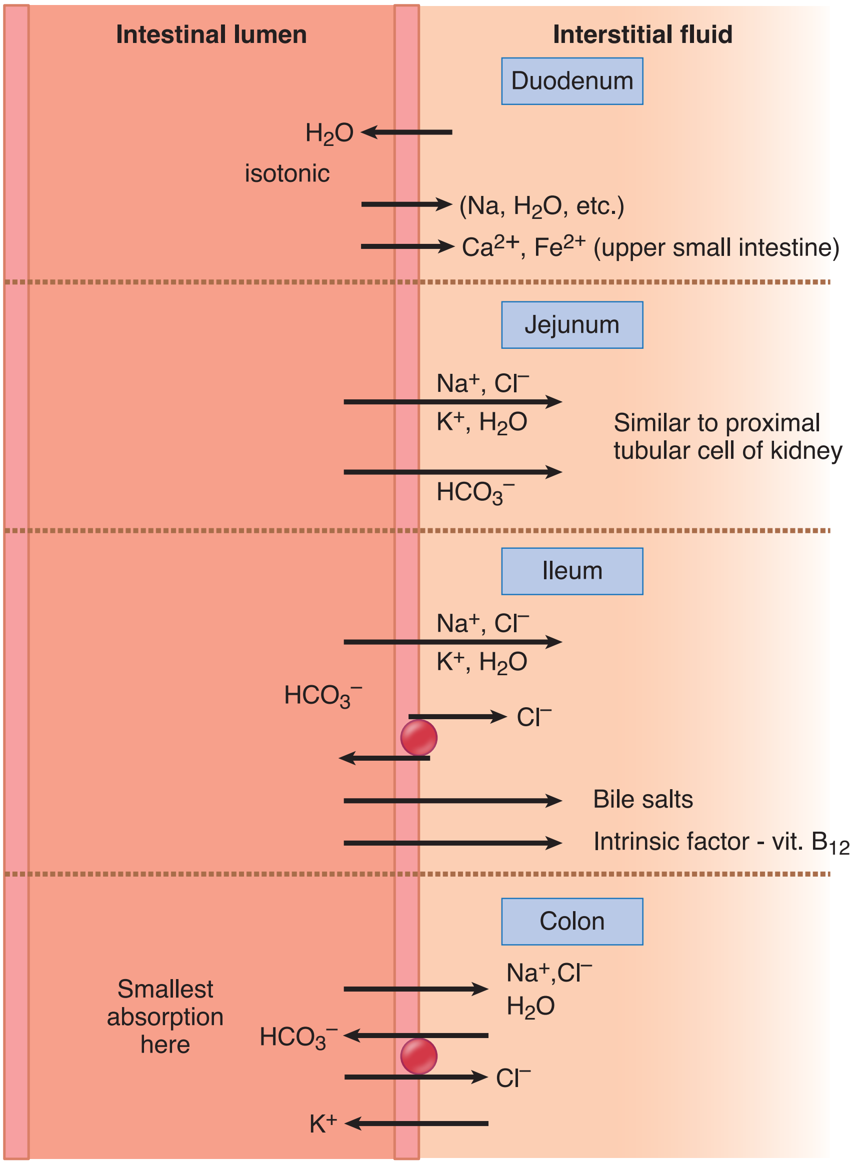


Figure VIII-3-4. Transport of Electrolytes

Diarrhea

Except for the infant where it can be hypotonic, diarrhea is a loss of isotonic fluid that is high in bicarbonate and potassium.

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